



INDIAN AGRICULTURAL  
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THE UNIVERSITY OF LIVERPOOL

ANNALS  
OF  
TROPICAL MEDICINE AND  
PARASITOLOGY

ISSUED BY THE  
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR J. W. W. STEPHENS, M.D.Cantab., F.R.S.

PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.

PROFESSOR WARRINGTON YORKE, M.D.

PROFESSOR B. BLACKLOCK, M.D.

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*Rev. V. Theobald*

**FREDERIC VINCENT THEOBALD**, M.A. (Cantab.), F.E.S., Hon. F.R.H.S., was born at Kingston-on-Thames, in 1868, and was educated at St. John's College, Cambridge.

He is a pioneer in zoological work, especially in relation to Medicine, Agriculture and Horticulture, and rendered a service of inestimable value to the study of Tropical Medicine when he prepared, for the Colonial Office and the Royal Society, his 'Monograph on the Culicidae of the World,' the first attempt ever made to classify all known species of mosquitos.

In addition to this, he has made many other contributions to the study of economic zoology, including 'The Insect and other Allied Pests of Fruit'; 'A Text-book of Agricultural Zoology'; 'The Insect Life'; and numerous Reports on Economic Entomology, issued by the South-eastern Agricultural College, Wye, during a number of years. For many years he has also devoted special attention to the Aphididae, and has increased our knowledge of this obscure group in many ways. He was also associated with Stephens and Fantham in the authorship of 'The Animal Parasites of Man.'

He was one of the first members of the Staff of the South-eastern Agricultural College, which he joined in 1894, and from 1900 till 1903 he was in charge of the Economic Zoology section of the British Museum. He received the Imperial Ottoman Order of the Osmanieh from the Sudan Government, in recognition of his scientific work; the Grand Medal of Isidore Jeoffrey St.-Hilaire of the Société Nationale d'Acclimatation de France; and, in 1913, the Mary Kingsley Medal of the Liverpool School of Tropical Medicine. He is a Member of the Honorary Committee of Management of the Imperial Bureau of Entomology (Colonial Office) and represented the Australian Commonwealth at International Scientific Congresses.





ANNALS  
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## THE ALAN H. MILNE MEDAL

This medal was struck to commemorate the late Alan H. Milne, C.M.G., the first Honorary Secretary of the School (1899-1917), and is awarded twice yearly on the recommendation of the examiners for the Diploma in Tropical Medicine.

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The following courses of instruction are given by the Liverpool School of Tropical Medicine each year:—

- (1) Two courses for the Diploma in Tropical Medicine, commencing on the 1st October and the 7th January. The D.T.M. examinations are held in December and March.
- (2) Two courses for the Diploma in Tropical Hygiene, commencing on the 7th January and the 24th April. The D.T.H. examinations are held in March and July.
- (3) A short advanced course, of one month's duration, in June.
- (4) Two courses in Veterinary Parasitology, commencing on 1st October and the 7th January.

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This Diploma shall be awarded only to candidates who possess a qualification to practise Medicine recognised for this purpose by the University, and who present satisfactory certificates of having attended approved courses of study, and pass the prescribed examination.

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1919	Maplestone, Philip Alan
1919	Oluwole, Isaac Ladipo
1919	Rustomjee, Khushhuyee Jamesidjee
1919	Sawers, William Campbell
1919	Thompson, Mary Georgina
1919	Turner, Gladys Maude
1919	Young, Charles James
1920	Adler, Saul
1920	Anderson, William Jenkins Webb
1920	Campbell, George
1920	Cobb, Charles Eric
1920	Cobb, Enid Margaret Mary
1920	Connolly, Evelyn Mary
1920	Fernandez, Daniel David
1920	Lim, Chong Eang
1920	McHutcheson, George Browne
1920	van der Merwe, Frederick
1920	O'Farrell, Patrick Theodore Joseph
1920	Renner, Edowo Awunor
1920	Vaughan, James Churchwill
1920	Waller, Harold William Leslie
1921	Allen, George Phillip Farmer
1921	Corfield, Charles Russell
1921	Hamid, Abdul

*Date of  
Diploma*

1921	Longhuist, Bell Wilmott
1921	Macvac, George Anthony
1921	Madan, Hans Raj
1921	Mulligan, William Percival
1921	Nixon, Robert
1921	Richmond, Arthur Stanley
1921	Shri Kent, Shamsheer Singh
1921	Skinner, James Macgregor
1921	Stewart, Robert Bell
1921	Thomson, Marion
1922	Bhatia, Jagat Ram
1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John
1922	Rieley, Stanley Desmond
1922	Rutherford, Gladys
1922	Stewart, Quinton
1923	Abelman, B
1923	Basu, Dharendra Nath
1923	Cruickshank, John Cecil
1923	Doherty, Winifred Irene
1923	Edghill, Winifred M
1923	Elsohn, John
1923	Fraser, N. D
1923	Lee, R.
1923	Pierce, E. R.
1923	Raja, Rajapuram
1923	Reid, C. B. B
1923	Richmond, A. E.
1923	Steven, J. B.
1923	White, Charles Francis
1924	Bilmotta, H. S.
1924	Carson, J. C.
1924	Chopra, B. L.
1924	Davis, B. L.
1924	Hardy, M. J.
1924	Jennings, C. B.
1924	Johnstone, F. J. C.
1924	Kenans, J. J.
1924	Lee, S. W. T.
1924	Macdonald, G.
1924	Maclean, G.
1924	Mathur, W. C.
1924	Mitchell, J. M.
1924	Owen, D. U.
1924	Palmer-Jones, Beryl
1924	Sankeralli E. J.
1924	Singh, H.
1924	Theron, Elizabeth M.
1925	Adams, Alfred Robert Davies
1925	Ashton, Frank Richard
1925	Ashworth, Esther
1925	Bamford, Charles Walker
1925	Beinashowitz, Jack
1925	Black, John
1925	Clark, George

*Date of  
Diploma*

1925 Coghlan, Bernard A.  
1925 Collier, Ivy  
1925 Crawford, E. J.  
1925 Cumming, Patrick Grant  
1925 Ellam, Mary Muriel  
1925 Fisher, Morris  
1925 Green, Frederick Norman  
1925 Grutu, M. S.  
1925 Hawe, Albert J.  
1925 Jafri, Z. H.  
1925 Johnstone, Elvy I.  
1925 Kerr, James R.

*Date of  
Diploma*

1925 Mackay, Donald M.  
1925 Mackay, E. K.  
1925 Makkawi, M.  
1925 Maldonado, Leopoldo Garcia  
1925 Mar, Severo Francisco  
1925 Mozoomdar, B. P.  
1925 Shah, Khwaja Samad  
1925 Skan, Douglas A.  
1925 Stone, Ernest R.  
1925 Terrell, C. G.  
1925 Tooth, Frederick  
1925 de Waal, Jacobus Johannes

# ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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Articles for publication should not exceed twenty-five pages of the *Annals*, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to:—The Editors, School of Tropical Medicine, The University, Liverpool.

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Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—‘According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.’ The references should be collected in alphabetical order of authors’ surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). ‘The spleen in malaria.’ *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). ‘Enlargement of the spleen in malaria’ *Journal of Pathometry*, Vol. I, pp. 1-20.

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## **Dr. Richard Caton**

We deeply regret to announce the death, at Haslemere, on January 2nd, of Dr. Richard Caton, Vice-Chairman of the Liverpool School of Tropical Medicine, and Pro-Chancellor of the Liverpool University.

Dr. Caton was not only a distinguished physician, but also a classical scholar and a social and municipal worker. He qualified as M.B., C.M., at Edinburgh University in 1867, proceeded M.D. in 1870, and afterwards became F.R.C.P. London, and received the honorary degree of LL.D. from the Universities of Edinburgh, Liverpool and Padua. He practised for many years in Liverpool and became President, and, later, Consulting Physician of the Royal Infirmary. He was Vice-President of the Council and subsequently Pro-Chancellor of Liverpool University, Dean of the Faculty of Medicine, and Professor of Physiology, receiving the title of Emeritus Professor on his retirement. In 1904 he was appointed representative of the University on the General Medical Council.

He was formerly Chairman of the Liverpool Committee for the Housing of the Poor, and of the Secondary Education Committee, was Lord Mayor of Liverpool in 1907-8 and was created C.B.E. in recognition of his work as Chairman of the West Lancashire Nursing Service Committee during the War. He also served as Chairman of the Liverpool Branch of the Classical Association, was a member of the Hellenic Society and Treasurer of the Board of Biblical Studies.

He was the author of several works of professional and archaeological interest, including 'The Temples and Ritual of Asklepios' and 'Imhotep and Ancient Egyptian Medicine.'

His death, whereby both City and University have sustained an irreparable loss, has occasioned deep sorrow throughout Liverpool.

# THE DEVELOPMENT OF *ONCHOCERCA VOLVULUS* IN *SIMULIUM DAMNOSUM*

BY

D. B. BLACKLOCK

(From the Sir Alfred Lewis Jones Research Laboratory, Freetown,  
Sierra Leone)

(Received for publication, 4 November, 1925)

PLATES I-IV

## INTRODUCTION.

The mode of transmission of various species of the genus *Onchocerca* has been a subject for speculation and experiment for many years. In the case of *O. gibsoni* and its allies in cattle particularly, much work has been done in order to discover the means of transmission, on account of the important economic results which necessarily follow the infection of meat with worm nodules. In the case of *O. caecutiens* in Central America great attention has been paid to the severe eye symptoms which the worms in the nodules have been credited with producing. The insect-borne theory of Onchocerciasis, the water-borne theory, and the theories of soil and direct contamination have each had their supporters. On the whole the tendency is to believe that an insect-borne theory best suits the facts. The following are some of the arthropods which have been suggested or experimented with for various species of *Onchocerca*. Doubtless there are others, but at present it is not possible to give a complete list.

*O. VOLVULUS*. *Glossina palpalis*, *Glossina longipalpis*, *Pediculus capitis*, *Pediculus humanus*, *Phthirus pubis*. *O. CAECUTIENS*: *Simulium samboni*, *Simulium dinelli* suspected by Robles. *O. GIBSONI*: *Haematopinus vituli*, *Trichodectes scalaris*, *Haematopinus eurysternus*, *Culiselsa vigilax*, *Culicoides subnitidus*, *Tabanus gregarius*, *Tabanus nigrotarsis*, *Stomoxys calcitrans*, *Lyperosia exigua*,

*Haematopinus tuberculatus*, *Musca domestica*, *Pycnosoma dux*, *T. cinerescens*, *S. elongatus*, *Silvius sordidus*, *Silvius* sp., *Tabanus lineatus*, and *T. near circumdatus*.

The investigation of which an account is given here was commenced in 1923-24 and continued in 1925, the whole of the work being done in the Konno district of the Sierra Leone Protectorate. In the first journey in this district the presence of cutaneous larvae corresponding in morphology to those of *O. volvulus* was observed while examining scabies (craw-craw) papules. The technique used was identical with that used by O'Neill in 1875, with the exceptions that a safety razor blade did service for a scalpel and a single section was made. O'Neill was examining the papules of Craw-craw and found skin microfilaria present in numbers; he gave a brief description of them and considered them to be the cause of this condition. The description corresponds so far as it goes with that of *O. volvulus* larvae. He describes his technique as follows:—'I find the readiest way to procure the filaria is to take between the finger and thumb a fold of the skin, so that the papule will be the highest point, then with a very sharp scalpel to slice off the epidermis, which may be discarded; now take another slice, which will remove the base of the papule and the cutis vera.'

In the Konno district not only were the larvae present in diseased and healthy skin in many persons, but the somewhat widespread prevalence of subcutaneous nodules was noted. The fact that the larvae were not found in the blood in persons whose skin was often heavily infected, made it probable that if any blood-sucking arthropod was the vector it would be one which in the process of penetrating to reach blood would rasp or tear the skin, with the result that the larvae would be dislodged into the wound and would then be taken up with the blood.

The Congo floor maggot *Auchmeromyia luteola* appeared well-adapted to dislodge larvae in the skin on account of the damage which it does before reaching blood. It is prevalent in the Konno houses, living in the mud floor; a number of Congo floor maggots were dissected without finding larvae in any of them.

At several villages in December, 1923, and January, 1924, it was noted that *Simulium damnosum* was biting in great numbers beside the small streams. They were a constant source of annoyance to

the boys who were then engaged in collecting snails for examination for cercariae and there was a continual slapping of legs to kill the fly, chiefly about the calves and ankles. In several cases blood could be observed trickling down the skin, especially when wet. The fact was noted, however, that after the fly had settled on the skin there was a considerable lapse of time before the person attacked felt any irritation and before the insect began to distend with blood. It appeared probable that this delay was due to the insects having some difficulty in piercing the skin, and this in turn suggested damage to the skin and the possible dislodgment of skin larvae if present. A hundred specimens of this species were dissected in order to see if any larvae could be discovered in the gut, but without any success. The investigation could not be continued at this time owing to pressure of other work.

On returning in 1925 it was determined to make a more extensive investigation in the Konno country in order to discover whether *Simulium damnosum* is capable of transmitting *O. volvulus*. The results of the preliminary blood and skin nodule investigations on the population are given here and also the results of the experiments so far carried out with a view to ascertaining the capacity of *Simulium* to act as vector.

## A. OBSERVATIONS ON HUMAN ONCHOCERCIASIS

### I. *Examination of human skin for the presence of O. VOLVULUS larvae.*

In proceeding north into the Protectorate, the skin of the villagers was examined in as many cases as possible for the presence of larvae and the occurrence of subcutaneous nodules. At a village called Tumbudu in the Konno district the conditions as regards skin infection with larvae, the occurrence of nodules in the subcutaneous tissue, and the prevalence of *Simulium* seemed favourable for the investigation and work was at once commenced here.

*Agamofilaria streptocerca*. This microfilaria described by Macfie and Corson in the year 1922 in natives of the Gold Coast was not discovered in the skin of the villagers at Tumbudu, where the only skin microfilaria found was that of *O. volvulus*.



*Number of persons examined.*

The number of natives whose skin was examined in one or more sites on the body was 123 ; of these persons fifty-two, that is over 42 per cent., had larvae of *O. volvulus* in their skin.

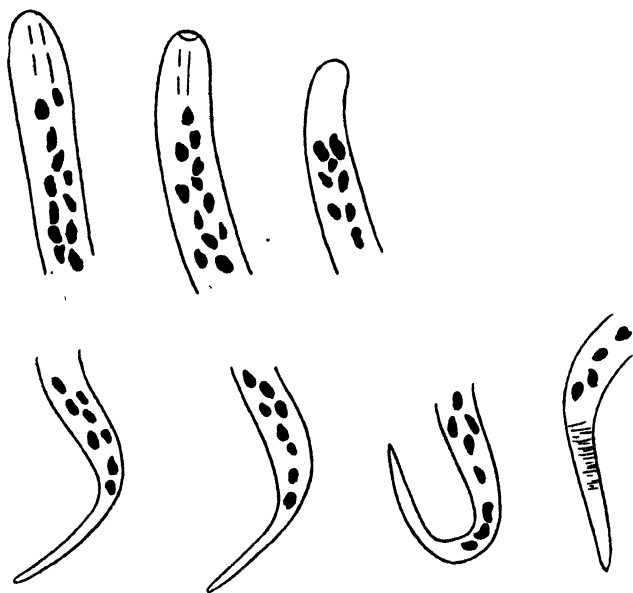


FIG. 1. *O. volvulus* forms in human skin.

Owing to the reluctance of females here to being examined by section of the skin, it was necessary to confine attention to males practically without exception. It is impossible, therefore, to make any comparison between the prevalence of *O. volvulus* larvae in the skin of the male and female sexes.

*Number of sections of skin examined.*

The total number of sections of skin examined from the 123 cases was 441 ; of these sections 102, that is over 23 per cent., were positive.

*Distribution of O. VOLVULUS larvae over the body.*

In a number of cases a more systematic examination of the body was possible ; pieces of skin were examined from four regions of the body, viz. : the scapular region, the loin, the thigh and the

ankle. Of ninety-three persons in whom this method was adopted forty-two, that is over 45 per cent., gave a positive result as regards the larvae of *O. volvulus* in one or more of the four sites. The findings in the different regions are tabulated below.

TABLE I.

Showing the distribution of *O. volvulus* larvae in 93 persons, each examined in four regions of the body.

Total persons examined	Total positive	Regions where <i>O. volvulus</i> larvae were present in the skin			
		Scapula	Loin	Thigh	Ankle
93	42	19	32	24	5

It is seen from the table that the waist region is that one of the four regions examined in which the larvae were most commonly found.

## II. *Condition of skin considered in relation to presence in, or absence from, it of O. VOLVULUS larvae.*

The observation made in the previous tour that *O. volvulus* larvae were frequently present in portions of skin which looked perfectly healthy was fully confirmed by the present series of examinations.

Special attention was paid to the condition of the skin in sixty selected cases referred to later. It may be mentioned here that the majority of these cases were members of the more important families of the village; that they were therefore better fed, better clothed, better washed and better housed than the average population. The condition of the skin in these cases was strikingly superior to that of the casual cases, who were often of a poorer labouring class. It was thus possible to find among these sixty cases seventeen whose skin was classed as very good, that is to say, that a complete examination of the body did not reveal any lesions of scabies, ulcers, or other obvious disease of the skin.

Of these seventeen cases having very good skins no less than eight had larvae of *O. volvulus* in sections made from various portions of the body. The youngest person having numerous larvae in a very good skin was twenty-four and the oldest fifty-one.

It appears evident, therefore, that the presence of *O. volvulus* larvae in the skin does not by any means always connote obvious

disease of the skin, even in people of advanced years ; whether this skin infection, if uncomplicated, does eventually produce obvious disease is a question which it appears impossible to answer at present.

III. *The existence of subcutaneous nodules considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

Sixty-eight cases were examined as to the presence of larvae in one or more skin sections, and subcutaneous nodules other than glands. The results are given in Table II.

TABLE II.

Showing the percentage of subcutaneous nodules other than glands in those having, and in those not having, *O. volvulus* in the skin.

Total examined	<i>O. volvulus</i> in skin	<i>O. volvulus</i> not in skin	Nodules present	Nodules absent	Percentage with nodules
68	30	—	12	18	40
	—	38	6	32	16

It is seen that only 40 per cent. of those having *O. volvulus* larvae in the skin had also subcutaneous nodules other than glands, while of those not having *O. volvulus* larvae in the skin 16 per cent. had subcutaneous nodules other than glands.

IV. *Condition of the lymphatic glands considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

The same group of sixty-eight persons was examined for enlarged glands ; in Table III are set out the figures obtained.

TABLE III.

Showing the percentage of enlarged glands in those having, and in those not having, *O. volvulus* larvae in the skin.

Total examined	<i>O. volvulus</i> larvae in skin	<i>O. volvulus</i> larvae not in skin	Enlarged glands present	Enlarged glands absent	Percentage with glands
68	30	—	8	22	27
	—	38	12	26	32

It is seen that only 27 per cent. of those having *O. volvulus* larvae in the skin had also enlarged glands, while of those not having *O. volvulus* larvae in the skin 32 per cent. had enlarged glands.

V. *The existence of subcutaneous nodules and the condition of the lymphatic glands considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

If we consider these sixty-eight cases from the point of view of the occurrence of either subcutaneous nodules or enlarged glands or both in cases with and without *O. volvulus* larvae in the skin, the figures given in Table IV are obtained.

TABLE IV.

Showing the percentage of persons with subcutaneous nodules or enlarged glands, or both, in those having and in those not having *O. volvulus* larvae in the skin.

Total examined	<i>O. volvulus</i> larvae in skin	<i>O. volvulus</i> larvae not in skin	Both glands and nodules	Either glands or nodules	Neither glands nor nodules	Percentage with either nodules or glands or both
68	30	—	2	15	13	57
	—	38	2	14	22	42

It will be observed that nodules or glands or both are present in 15 per cent. of those who have larvae in the skin, in excess of those who have not larvae in the skin. The margin is probably not sufficient to permit of arriving at any general conclusion as to the relationship between glands and nodules on the one hand and skin infection with *O. volvulus* larvae on the other.

VI. *The condition of the eyes considered in relation to the presence in, or absence from, the skin of O. VOLVULUS larvae.*

In view of the fact that profound ocular changes are stated to be due to the action of *O. caecutiens* in Central America, attention was paid to the eyes of certain cases which came for examination.

In a series of twenty-three unselected cases the vision was tested and the eyes were examined externally for evidence of disease ; in the same cases the skin was examined for the presence of *O. volvulus*

larvae. The tests of vision utilised for these illiterate natives were two, for near vision the threading of a sewing needle, and for distant vision the counting of a number of pieces of stick held at different distances. The only abnormalities found were :—slight exophthalmos without visual defect, two cases ; pterygium coupled with defect of vision approximately R 3/6 L 1/6, one case ; chronic blepharitis oedema of sclerotic and pterygium coupled with defect of vision R 3/6 L 2/6, one case ; history of discharge from eyes at intervals for one year, one case.

The results of the examination of the eyes and of the skin are set out in Table V.

TABLE V.

Showing the results of eye examination and of skin examination for *O. volvulus* larvae in 23 unselected cases.

Total examined	Skin examination		Eye examination	
	Larvae present	Larvae absent	Eyes normal	Eyes abnormal
23	11	—	7	4
	—	12	11	1

At first sight it might appear that there were more abnormal eyes among persons having *O. volvulus* in the skin than in those not having these larvae in the skin. It appears possible, however, that this apparent difference may to some extent be explicable on the ground of age. The persons who had larvae in their skin in this series happened to be much older than those who had no larvae in the skin. Thus the average age of the seven having normal eyes and larvae in the skin was forty years and that of the four having abnormal eyes and larvae in the skin was forty-seven years ; whereas the average age of the eleven having normal eyes and no larvae in the skin was twenty-six and the age of the case having abnormal eyes and no larvae in the skin was twenty-five.

A series of thirty-eight cases selected in age periods gave a different result as seen in Table VI.

TABLE VI.

Showing the result of eye examination and of skin examination for *O. volvulus* larvae in 38 selected cases.

Age	Total examined	Skin examination		Eye examination	
		Larvae present	Larvae absent	Eyes normal	Eyes abnormal
	38	18	—	18	0
	—	—	20	19	1
0 to 10	8	0	8	8	0
11 to 20	3	1	2	1	0
21 to 30	9	6	3	6	0
31 to 40	6	3	3	3	0
41 to 50	5	4	1	4	0
51 over	7	4	3	4	0
TOTAL ...	38	18	20	37	1

In this series only one case had abnormal eyes, a condition of pterygium with history of discharge from the eyes periodically: vision good.

No evidence of such conditions as irido-cyclitis, keratitis punctata, or panophthalmitis was obtained in either of these series. On the contrary the general appearance of the eyes and the visual acuity even in the older cases was excellent. In this locality, therefore, it was not possible to obtain evidence which would justify any etiological association between disease of the eyes or defects of vision and the infection with *O. volvulus* as determined by skin and nodule examination.

## VII. *Character of the subcutaneous nodules.*

These varied from a smooth, soft, just palpable nodule to a large, irregularly nodular, hard mass of the size of a large walnut. The first type was usually found in the region of the great trochanters, on the ribs (Case 50), and on the scalp; the second type was generally closely associated with joints, more especially the elbow and knee joints; neither type was usually adherent to the skin, but in some cases, especially over the great trochanter region, the skin over the nodule was thickened, partly adherent, and polished on the surface from pressure.

Pain was frequently complained of, and some persons who had nodules on both trochanter regions had resorted to the use of a circular ring-pad resembling a magnified bunion pad to enable them to sleep on the side.

Cases were examined in which larvae were found in the skin without palpable nodules being present; cases were found where *O. volvulus* larvae were present in the skin while the nodules which were present gave negative results on puncture; cases were found where *O. volvulus* larvae were present in the skin and also in nodules; there were no cases in which, although no larvae were found in the skin, puncture of a nodule gave a positive result.

### *Juxta-articular nodules.*

If the region had not been one in which *O. volvulus* was present, several of the cases seen would have been classed as juxta-articular nodules.

The examination of puncture fluid by no means always cleared up the diagnosis. In this connection special reference may be made to two cases.

CASE 59. This was a man of fifty-five years of age, whose skin was apparently normal except over the knees and elbows, where it was much wrinkled; the eyes were free from disease and the visual acuity was good; the blood was negative. On both sides the inguinal glands were enlarged. He had the following nodules:—

Near R trochanter	...	2	each about	$1\frac{1}{2} \times 1\frac{1}{2}$	inches
L       ,,	...	2	,,	$1\frac{1}{2} \times 2$	,,
R elbow	...	1	,,	$2 \times 2$	,,
L       ,,	...	1	,,	$2 \times 1$	,,
L knee (head of Tibia)	1	,,		$1 \times 1$	,,

These nodules were irregular in shape and had accessory small nodules at the margins. They were hard, fairly mobile under the skin, and non-adherent. They gave the impression of resulting from the fusion of several nodules.

Puncture of the left elbow nodule resulted in a flow of amber-coloured fluid oozing slowly from the needle. Puncture of one right trochanter nodule produced a few drops of serous fluid; the puncture of the remaining nodules gave small amounts of an opalescent fluid with light brown granules in it. In addition to each nodule being punctured once, two of the nodules, one on the right trochanter and one on the left trochanter, were punctured a second time in a different place. None of these nine punctures revealed the presence of either eggs or larvae. It would perhaps have been legitimate to conclude that these nodules were not connected with infection with *O. volvulus*. Such a conclusion, however, appeared less justifiable in view of the fact that the examination of skin sections revealed a particularly heavy infection with *O. volvulus* larvae in the scapular, loin, and thigh regions, although not in the ankle region; gland puncture was not permitted.

CASE 51a. Here the nodules were not so numerous but they were of a similar character and gave an even more interesting result. The nodules were :—Right femur internal condyle, two; right trochanter region, two. Puncture of the four nodules gave a negative result for three and a positive result for one, namely, one of those over the condyle of the femur. The blood was negative, but the skin of scapular, loin, thigh and ankle regions was heavily infected with larvae of *O. volvulus*.

## B. OBSERVATIONS ON *SIMULIUM DAMNOSUM*

### A. GENERAL.

#### *Hour of day at which biting.*

Observations were made at various hours of the day in order to ascertain at what hours the fly was prepared to feed in this locality and whether any particular hours were preferred by it. It was found that individuals were not captured biting earlier than 6 a.m. and that only the latest stragglers were biting as late as 6 p.m. In the



middle of the day the fly would not go far in order to bite, not even five yards as a rule. The flies were found biting, however, in bright sunny weather at any hour of the day, provided they had only a yard or two to go from their shelter. Even on sunny days they were thus captured at any hour of the day from 6 a.m. to 6 p.m. On dull days without rain they bit freely at any hour of the day and a slight shower did not reduce the catch materially. On wet days, however, with heavy rain, they were not found biting, and it was noted also that soon after a heavy rain there was a considerable lapse of time before they would again come out to bite. All these records were made close to the edge of water, usually flood water from the large river or near small streams.

It was frequently noted that during a period which gave a good capture there were intervals in which the fly was not biting. They thus appeared capricious at times when no alteration was noticeable in the temperature or light ; the fly was sought out in places where it was known to exist and the collectors encouraged them to bite by disturbing their resting place in the long grass and bush and submitting quietly to their attacks. It was noticeable that the fly did not come out to attack as it was observed to do in the month of December in another locality.

### *Length of life in captivity.*

Flies captured while just commencing to feed and kept in tubes of various sizes without food, and with or without moisture supplied, whether kept in the dark or in the light, failed to survive more than three days.

Various experiments were made in order to discover some means of keeping wild flies alive in captivity, but the results were disappointing ; the containers were covered in each case with gauze of 50-55 meshes to the inch ; this, in the case of solid containers such as those of tin and wood, made the inside dark. The containers used were of glass, metal or wood, and were of various sizes and shapes. Foods of various kinds, e.g. moist raisin, fresh banana, oranges, and particles of meat were supplied ; the condition of moisture was varied by adding damp blotting paper, damp sand or free water. Efforts to reproduce the conditions of nature consisted in placing small plants with the soil in which they were growing in several of the containers.

In spite of the efforts made, the longest period of survival in a total of 268 flies was ten days, and that a solitary instance. Individual specimens lived for eight and seven days, but by far the majority were dead within four days. On the whole, it may be said that dry glass cylinders and large test tubes with raisin as food gave better results than did either wood or metal containers, but the cases of survival were few and far between.

### *Effects of sunlight.*

Fully-fed flies exposed in test tubes immediately after the feed to the full glare of sun just before noon died in twenty to sixty seconds after a period of violent excitement. Partially-fed flies survived for one to two minutes.

### *Feeding in captivity.*

In spite of numerous attempts made, using various expedients such as moistening the skin, smearing with plant juices, serum or blood, no fly was ever induced to bite the human skin, either white or black, in captivity. Flies were observed to sit on moist raisin and appeared to be attempting to feed, but they did not engorge; they apparently took up a little water from time to time from the muslin at the edge of the moist food.

### *Time taken to feed and attitude in feeding.*

1. *On human beings.* The time required by the fly to feed to repletion was noted on several occasions, when flies were observed to settle and bite and fly away unmolested. It was found that the time varied from one-and-a-half to five minutes. There is a considerable interval after the fly alights and begins to operate on the skin before any distension of the abdomen is visible; in flies captured at this point and dissected, the gut frequently contained cellular debris; the majority of the time was occupied in getting down to the blood level and after this was reached distension was a rapid process. The attitude at the commencement was with the body parallel to the skin surface, the wings flat and almost overlapping; by the time the abdomen was nearly distended the position was altered so that the whole body was at an angle to the skin with the head close down to it and the tip of the abdomen well raised from the surface.

They were easily disturbed in the early stages of their feed, but after they had reached blood and commenced to engorge, they were not disturbed by placing a test tube over them and went on feeding till distended, and they were thus easily captured. The extent to which these flies engorge is remarkable and the abdomen distends visibly owing to the large accumulation of blood in the mid-gut ; in specimens dissected at periods varying from half-an-hour to

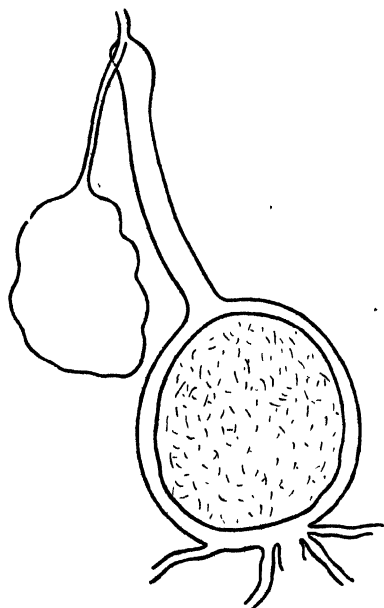


FIG. 2. Midgut of *Simulium damnosum* distended with human blood.

six hours after feeding, the blood was always found in this organ and never in the thin membranous structure which apparently corresponds to the food reservoir of tsetse-flies. The blood forms a globular mass which retains its form even after being pressed out of the cut anterior end of the gut ; the blood mass is semi-solid, sticky in consistency, and difficult to disintegrate by needles, soon after the feed ; altered blood was found in small amounts up to four days after feeding. The food reservoir was usually found to be full of a clear glairy fluid after the expression of which the thin walled sac collapsed.

2. *On animals.* Only a few experiments on animals were made, employing a sheep, two young goats, and a dog. These were taken at various times to the spots where flies were being captured biting human beings. The fly was not seen to bite the animals when these were walking about. It was possible, however, by placing the young goats on their backs to observe that two flies bit them and engorged with blood in the regions of the mammary gland and axilla. The time taken to engorge did not exceed the time found for human beings, and the degree of engorgement was the same.

*Effects of rain and immersion in water during feeding.*

It was found that a coarse spray of water applied to the fly while biting caused it to relinquish its hold. In the same way the direct impact of rain drops on the feeding fly dislodged it. If, however, the limb on which the fly was feeding were immersed gently in water the fly very frequently did not relinquish its hold.

1. Fly biting on leg just above ankle; whole foot and ankle gently immersed in still water so as to cover fly to a depth of two inches; fly remained and continued biting under water and swelled out; on removing foot gently the fly was still attached but almost immediately flew off.

2. In a similar experiment, the fly remained immersed for five minutes but was loose on bringing the foot to the surface.

3. A feeding fly was immersed for three minutes; on removal of the foot was still feeding.

4. A fly biting on dorsum of foot was immersed; in three minutes came up and stuck to hairs on leg just below surface of water; thirty seconds later it suddenly shot up to the surface, circled about rapidly for a few seconds on the surface and then flew off.

The fly appeared to have attached to it bubbles of air when it came up to the surface, and it made rapid gyrations on the surface, seemingly until these bubbles burst, before flying off.

*Cutaneous and general reactions after bites.*

When a fly settles, which it does without sound, and commences biting, nothing is noticed as a rule if the attention is otherwise occupied; in a large number of experimental observations the

person being bitten was quite unaware of it, even when the fly was engorging with blood, if the attention were otherwise engaged ; in other cases where the attention was directed entirely with the object of detecting when the fly was biting the initial stages of the bite were not noticed, and irritation was first felt when the fly commenced to draw blood.

Certain persons, few in number, failed to observe that they were being bitten even when their attention was directed to detecting bites, and the flies would engorge and go off unperceived. There is great variation in sensibility in this respect, but when flies are biting in any number even the most unobservant becomes aware of it.

The first thing noticed is a very slight scratching sensation which goes on for about a minute or more ; this is followed by a slightly painful pricking sensation and this finally by an intense irritation which comes on just as the fly is ready to move away.

If the affected part is immediately scratched the irritation quickly passes off, the lesion made by the fly having been enlarged by the scratching. In natives, even if scratching was prevented little local reaction could be seen on the skin ; they appeared to be immune ; on the skin of a white person examined, the reaction on the unscratched skin consisted of a white raised wheal about 5 to 8 mm. across with a red point in the centre ; intense irritation was felt for ten minutes, and subsequent slight irritation for about two days.

In cases where numerous flies bit close together on the skin, especially when it was damp, the lesions would occasionally ooze somewhat freely and the blood droplets would coalesce and form a drop which trickled down. Non-biting flies were seen to settle on the skin and feed on the blood in such cases.

### *Prevalence of fly.*

Some indication of the numbers of fly actually biting when given the opportunity in their haunts at this season may be gained from the following figures ; each number given represents those caught on a single individual ; no males were captured.

On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 73
On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 58
On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 55
On 31.7.25, bright and sunny, 10.30 a.m. to 1.30 p.m.	...	1 boy caught 10

On 31.7.25, bright and sunny, 8 a.m. to 2 p.m.	...	...	1 boy caught	80
On 1.8.25, dull, sunless, 7.30 a.m. to 8.30 a.m.	...	...	1 boy caught	25
On 1.8.25, dull, sunless, 10 a.m. to 4 p.m.	...	...	1 boy caught	46
On 24.8.25, sunny, 8 a.m. to 10 a.m.	...	...	1 boy caught	24
On 25.8.25, dull afternoon	...	...	1 boy caught	30
On 25.8.25, dull afternoon	...	...	1 boy caught	30

It is seen that here we have a fly which is so numerous and bites so freely that any individual during the course of the day may receive a very large number of bites. Even although only a small percentage of those which bite acquire or transmit a disease virus it is clear that the very large numbers would make the transmission probable, provided that subsequent biting occurs after such an interval as would permit of the development to an infective stage of the virus in the fly.

#### *Situation on the body of bites received.*

In considering the part of the body bitten it may be stated that the experiments were carried out in those places where flies were being captured, that is, in fairly open areas without much high shade and in grass about three to six feet high with a few shrubs among it. A small portion of the low grass was beaten down and the person stood or sat in the clearing. It was found, if a person stood erect with feet, legs, and thighs exposed to bites in such open situations, that about 80 per cent. of the total bites were received below the level of the knee. The fly attacked a portion of the skin which was in shade and a favourite site was just below the bulge of the calf or below the malleoli. When the person sat or squatted in the cleared area with the skin exposed, the buttocks and loins were chiefly attacked; a person standing among the grass before it was trampled down was bitten wherever the grass reached on his body. The fly did not, as a rule, leave the shade afforded by the grass and the lower parts of the trunk, and attack the face, neck and shoulders. It was observed that while sitting on a camp-stool in the centre of the small cleared area, the arms, neck and face, although exposed, were never bitten during an hour, while persons standing were bitten below the knee and those sitting on the ground were bitten on the loins, buttocks, and legs.

*Habits as regards following human beings.*

Native accounts describe this fly far from water and pursuing the rice harvesters on dry farms; little evidence of following was obtained however, during the period of investigation. The nearest point to the rest-house at which fly could constantly be captured was 150 yards away. There was a steady traffic of persons to and from this spot at the edge of the river, yet in the whole period only half-a-dozen flies were caught biting in the rest-house compound. Apart from the fact that persons passing through the position occupied by the fly were not followed, there is also the fact that the bush extends from the spot right up to the rest-house compound. It is seen, therefore, that little or no movement of the fly through the bush was taking place here at this time. In experimenting on this question it was found that if the fly-collector was standing or sitting in a small cleared area at the edge of the long grass, other persons could stand on the path at a distance of only five yards from this spot and hardly ever receive a bite; at two yards the bites were more numerous, more especially if the skin was moistened with water. There was thus no evidence here at this period of the year of following of human beings by the fly from those of its permanent sites, near the water, which were tested.

*Types of country in which fly was found biting.*

The proximity to water was the essential feature noted. In Plate II, fig. 3, is shown a river of some size on the shelving banks of which, where they were grass-covered and provided with shade from trees and shrubs, the fly could be found biting at almost any hour of the day except in actual rain or immediately after a heavy rain.

On the opposite side (Plate II, fig. 4) of this river the banks are low, and overflow was constantly occurring with each rain. Among the grass at this side the fly was constantly to be found also. The collector simply walked in from the path at any point, trod down some of the grass, beat the standing grass with his hands and, taking off his clothes, sat down. In a few minutes dozens of flies would be round him trying to select a shady spot on his skin on which to feed.

The wet rice farms also were favourite spots ; most frequently these lay on each side of small slow streams and here the rice was growing on sodden ground. There might, or might not, be overhead shade, but if not, the fly bit very low down. Some collectors found that by first wading through water and then offering their skin to the fly, they received more bites.

*Habits of people in relation to bites of Simulium.*

People bathing in the rivers are not frequently bitten, but those who stand on the banks of the river suffer severely. The farming operations involve both sexes ; the bush is cleared by the men, a certain amount of weeding is done by the women and both take part in cutting the rice. This process involves walking through the growing rice and cutting the tops off, the straw being trampled under foot. The clothing worn in these operations is reduced to a loin cloth, or this and a very short shirt may be worn. The places on the body which would therefore most usually get bitten are the regions below the level of the top of the growing rice, that is, from the waist downwards. Young children are employed to frighten the birds, but are perched on high pedestals erected on the farms.

In the Konno country all go to the waterside to defaecate. The waterside area is in close bush and the latrine is normally the edge of a small stream. Here the fly has an excellent and frequent occasion to bite about the waist region of both sexes. Both the habits of the fly as seen here and the habits of the people tend to produce the same effect, namely, that it is the region from the waist down that is most attacked ; possibly males are more exposed to its attacks than the females during their farming operations.

*Native names.*

Some of the tribal names for Simulium in Sierra Leone are :—

Konno	...	Mooli or Mooyee
Timne	...	Mapus
Mendi	...	Mawwee
Lokkoh	...	Poondée gootena, i.e. short mosquito (Poondina = mosquito).
Susu	...	Koondée
Mandingo	...	Mootee



## B. DISSECTION OF WILD SIMULIUM

### *Gut of fly.*

Boys were taken to places where the fly had been observed biting and instructed to catch each fly after it had begun to feed on the skin. The flies were then dissected in order to see whether any of them were ingesting *O. volvulus* larvae, the gut alone being examined. Of a total of 780 flies which were so dissected twenty, that is, 2.6 per cent., had in their gut larvae which appeared in the fresh state to be *O. volvulus* larvae, and which on staining showed the morphological characters of this larva. The larvae were conspicuously active in the gut contents of the fly, much more so than when dissected out of pieces of skin in a drop of water on a slide. The blood was found soon after a meal to be agglomerated into an amorphous-looking, spherical mass, and the larvae could be seen actively moving chiefly between the anterior surface of this mass and the wall of the mid-gut; they were active in flies which had died six, twelve, and even eighteen hours before dissection.

It was, therefore, proved that *Simulium* is capable of taking up, in feeding, larvae having the character of the larvae of *O. volvulus*, and further, it was proved that the larvae, having been ingested, not only retained vitality but definitely increased in the activity of their movements; the latter fact pointed to the possibility of the larvae undergoing further development in the fly.

### *Thorax of fly.*

Flies which had been captured in the same manner by the boys were examined as to the occurrence of developmental forms of larvae in the thorax. Of 1,320 flies in which the thorax was dissected developmental forms of larvae at different stages were found in fifteen, that is, over 1 per cent. This investigation showed that nematode larvae of some kind were capable of development in the thoracic tissues of *Simulium*.

### *Head of fly.*

This was dissected in the case of 1,140 flies caught by the boys; in no case was a larval form found actually in the head, although one form was partially embedded in the head and was pulled out of the anterior thoracic region with the head.

*Identity of the thoracic forms.*

While, therefore, there was evidence that development of some larvae was capable of proceeding in *Simulium* there was no evidence that the developing forms were part of the life-cycle of *O. volvulus* ; the only suggestive points were that, as previously stated, it had been proved that *Simulium* could readily take up larvae morphologically identical with this in feeding, and that when in the insect's gut the larvae showed increased activity and good viability.

In order to obtain confirmatory evidence bearing on this point the following examinations were carried out, the object being to determine as far as possible by a process of exclusion what the larvae were which were present and developing in the thorax of wild *Simulium* in this district.

- I. Examination of day and night cutaneous blood of selected human beings.
- II. Examination of day cutaneous blood of unselected human beings.
- III. Examination of day cutaneous blood of domestic animals.
- IV. Examination of the skin of domestic animals for microfilariæ.

*Examination of day and night cutaneous blood of selected human beings.*

Sixty persons, all males, were examined for microfilariæ in the cutaneous blood of the ear at noon ; a thick film for each person was examined. It was intended to examine the same persons at midnight, but there was some difficulty about persuading them, so that only forty-six of them could be collected for the midnight examination.

The cases were selected so as to give ten persons in each of six age groups and the results obtained are set out in Table VII.

A single sheathed microfilaria present at noon in the film of one individual in the 41-50 age group proved to be *Microfilaria bancrofti*, the same individual at midnight being found to have numerous microfilariæ of this species present in the cutaneous blood. All of the other persons who had microfilariæ in the cutaneous blood at midnight were also infected with this parasite, no other being found.

This examination, therefore, revealed no infection with either

TABLE VII.

Showing the findings in the cutaneous blood of 60 males at noon and 46 males at midnight.

Age period	Noon		Midnight	
	Number examined	Number having microfilariae	Number examined	Number having microfilariae
0 to 10 ... ..	10	0	10	0
11 to 20 ... ..	10	0	3	0
21 to 30 ... ..	10	0	9	4
31 to 40 ... ..	10	0	7	2
41 to 50 ... ..	10	1	8	3
Over 50 ... ..	10	0	9	4
TOTALS ... ..	60	1	46	13

*Acanthocheilonema perstans* or *Loa loa*, while it demonstrated a moderate infection of this sample of the population with *F. bancrofti*.

Examinations of additional persons at a late hour of the night were not possible owing to the reluctance of the villagers to attend. Additional cases were, however, examined at various hours of the day from soon after 6 a.m. to nearly 6 p.m., as shown in the following Table

TABLE VIII.

Examinations of day cutaneous blood of 83 unselected human beings.

Time	Number examined	Infected with microfilariae
6 to 7 a.m. ... ..	4	0
7 to 8 a.m. ... ..	9	0
8 to 9 a.m. ... ..	7	0
9 to 10 a.m. ... ..	9	0
10 to 11 a.m. ... ..	11	0
11 to 12 noon ... ..	21	0
12 to 1 p.m. ... ..	5	0
1 to 2 p.m. ... ..	5	0
2 to 3 p.m. ... ..	5	0
3 to 4 p.m. ... ..	4	0
4 to 5 p.m. ... ..	2	0
5 to 6 p.m. ... ..	1	0
	83	0

It is seen that in this series also neither *A. perstans* nor *Loa loa* was found.

As a result of this examination it appeared improbable that the larva found developing in the thorax of *Simulium* was that of either *A. perstans* or *Loa loa*. It might be that of *F. bancrofti*, as the popula-

tion harboured this parasite in a fair proportion of cases. Against this, however, we have the biting habit of the fly, which was found biting here at this season only between the hours of 6 a.m. and 6 p.m. It appears possible, however, that the fly might become infected with this parasite even if its biting was entirely diurnal, because *F. bancrofti* larvae were seen once at mid-day in one case and possibly were present in the cutaneous blood of other cases in numbers which, although too small to be revealed by the examination of a single thick film, might yet be taken up by the fly in biting.

### III. *Examination of day cutaneous blood of animals.*

No cattle or horses were present in the village of Tumbudu nor were any seen at any of the sites at which the boys were engaged in collecting flies.

There were a few sheep and goats and one dog in the village, and blood from the ear of the majority of these was examined in fresh film. Thirty-one sheep and nine goats were examined in this way without the discovery of any microfilariae in them. An examination of these animals was all the more necessary as it was proved that *Simulium damnosum* is capable of engorging upon goats at least.

### IV. *Examination of the skin of animals for microfilariae.*

The portion of the tip of the ear of each animal from which blood was being taken was kept and teased up with needles in a drop of water on a slide and examined for microfilariae; in no case was a microfilaria found.

The general effect of the results of these examinations was to give the impression that whatever the larva was which was found developing in the thorax of wild *Simulium damnosum*, it was probably neither that of *A. perstans*, *L. loa*, or *Ag. streptocerca*, nor of a filaria of cattle, horses, goats, or sheep. The probabilities rested then between *F. bancrofti* and *O. volvulus*.

One of the direct results of this series of examinations was that it was rendered possible to make an advance by attacking the problem in a somewhat different way. There now became available certain persons of whom it was known that they did not present

larvae of *L. loa*, *A. perstans* or *F. bancrofti* in their cutaneous blood, and in whose skin only *O. volvulus* could be demonstrated. The cases which fulfilled these conditions were re-examined and two of them were selected as presenting the most intense infection of the skin with *O. volvulus*. These cases were then used to replace the previous fly-collectors; they were taken to the chosen site and each was attended by a boy whose work consisted in collecting flies off one of these two cases. The process of collecting was carefully supervised in order to avoid the possibility of the flies being captured on any other person than the two special cases.

The immediate effect of adopting this procedure was that whereas the gut infections had hitherto been 2.6 per cent. in 780 wild flies caught on the unselected boys engaged in collecting, it rose at once to 17 per cent. when the flies dissected were all collected from these two cases. At this stage the less heavily infected case was discarded and the remaining case was alone exposed to the bites of the fly, and in successive experiments this man was clothed so as to allow only certain areas of skin to be bitten.

First of all, he was clothed so as to expose to the fly only the region from the loins upwards; as a result of this the percentage of fly infection in the gut rose to 28. In the next experiment all the body was protected except the waist and hips; flies captured feeding on this area showed a gut infection percentage of 59.

Finally the case was exposed to the fly in such a way as to allow biting only on a narrow band of skin four inches wide round the hips, having the subcutaneous nodules, which were present near each trochanter, in the centre of the band. In this last experiment the percentage of gut infected flies rose to 80.

The results are shown in Table IX; the fact that the area of body exposed was more and more restricted necessarily had the result that smaller numbers of flies could be collected from the case. The blood of these cases was examined frequently, usually just before starting out to collect and immediately on return. No blood microfilariae were found other than, on two occasions, *Microfilaria volvulus* itself.

TABLE IX.

Showing increase in percentage of gut infection in *Simulium damnosum* by allowing it to feed on selected cases and on special areas.

Feeding on	Number of flies	Gut infections	
		Number	Percentage
Unselected boys ... ..	780	20	2.6
Cases 47 and 50 (whole body) ... ..	41	7	17.0
Case 50 (loins upwards) ... ..	54	15	28.0
Case 50 (waist and hips only) ... ..	29	17	59.0
Case 50 (on band of skin round hips with nodules in centre) ... ..	20	16	80.0



FIG. 3. Edge of blood mass in anterior part of midgut of *S. damnosum*, with larvae of *O. volvulus*.

This series of experiments not only served to confirm, amply, the observation that *Simulium* can take up the larvae of *O. volvulus* from the skin, but also showed that when selected areas of heavily-infected skin were exposed to it very large numbers of larvae were ingested. In some flies between one and two hundred *O. volvulus* larvae were counted in the gut. It seems probable that when this species of *Simulium* feeds on an area of skin affected with *O. volvulus*

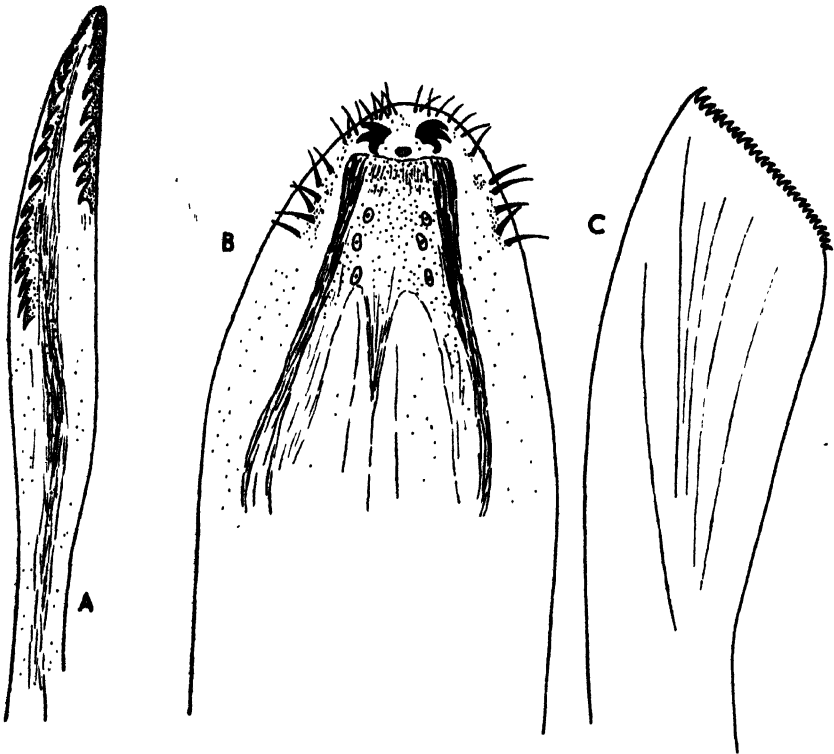


FIG. 3A. *Simulium damnosum*. A.—Maxilla; B.—Labrum epipharynx; C.—Mandible.

larvae it invariably takes up some of them. Corroboration of this view is found in the study of the mouth parts and the ragged type of lesion which the biting parts of this species inflict on the skin.

At the same time the experiments demonstrated the fact that it is not necessary that the larvae of *O. volvulus* should be circulating in the blood in order to permit of their being taken up by a blood-

sucking insect. The repeated examination of the cutaneous blood of these cases during the period proved conclusively that the larvae were very rarely found in a drop of blood taken from the skin ; even on the occasions on which they were found in such a drop it was not feasible to exclude the possibility of their having been derived from the skin ; in fact, that this was the source of those larvae appears highly probable.

*Development of O. VOLVULUS larvae in Simulium.*

The next step was to endeavour to ascertain whether the *O. volvulus* larvae which were so readily and constantly taken up by *Simulium damnosum* would proceed to develop in the body of this insect provided it was kept alive after feeding on infected skin. Incidentally it would be of interest to discover whether the developmental forms, if found, would correspond to those forms which had already been found in a percentage of the wild flies captured biting on unselected persons. The experiments were as follows :—

(1) Ten flies which had fed on a boy infected in the loin skin, but which were collected from any portion of his body, were kept until they died and were dissected. In one fly which died after thirty hours from the time of feeding, development forms of an early stage were found in the thorax. The other nine, which had all died before three-and-a-half days, proved to be negative.

(2) Large numbers of flies were captured on the two cases (47 and 50) feeding on any part of the body. Of the total of flies so captured very few survived more than a few days. Twenty-two, however, were dissected soon after their deaths and in this total, seven, i.e., 32 per cent., proved to be infected in the thorax.

(3) Twenty-two flies which were captured biting on Case 50, on the band of skin four inches wide, with the nodules in the centre, as described previously, were kept alive. Of these twenty-two flies, which were all dissected at periods later than two days after their feed, eighteen, or 82 per cent., proved to be infected in the thorax. In Table X are set out the percentages of thorax infection obtained in these experiments.



TABLE X.

Showing increase in percentage of thorax infection in *Simulium damnosum* by allowing it to feed on selected cases and on special areas.

Feeding on	Number of flies dissected	Thorax infected	
		Number	Percentage
Unselected boys ... ..	1,320	15	1'1
One infected boy (all over body) ... ..	10	1	10'0
Cases 47 and 50 ... ..	22	7	32'0
Case 50 (nodule band) ... ..	22	18	82'0

From a consideration of these experiments on gut and thorax infection it appeared that when flies were given an opportunity of feeding on known infected skin, the percentage of gut infections rose to a very high figure, such as was never approached by flies captured on casual collectors; again, such flies when kept alive showed a percentage of infection with developing forms in the thorax which was far in excess of anything obtained from the dissection of wild flies fed on casual collectors. In view of the process of exclusion of other larvae, both human and animal, which preceded the last part of this investigation, there appears no doubt that the forms which were developing in the thorax of such flies as fed on the selected cases were derived from larvae ingested from the skin of these cases by the fly and that they were, in fact, the developmental forms of *O. volvulus*.

#### *Examination of other insects.*

Reference has already been made to the dissection of the Congo floor maggot, *Glossina palpalis*, which was present but not plentiful in the bush adjacent to the Moendi River, near Tumbudu, was captured on five occasions when feeding on Case 50. Of these five tsetse-flies, two had larvae of *O. volvulus* in the gut, one fly having one and another fly having two. The flies were dissected within an hour of feeding but the larvae were motionless and appeared to be dead. From these few specimens it is evident that *G. palpalis* is capable of taking up the larvae of *O. volvulus*, but it does not appear

that it can take up any numbers comparable with those taken up by *Simulium*. Further, whereas the larvae in the gut of *Simulium damnosum* became more active, the reverse was the case for the larvae discovered in the gut of the two infected tsetse-flies.

*Tabanus* sp.\*

Forty-seven specimens of this fly were dissected in the gut and thorax without the discovery of any larvae. These flies were very common at this place but the natives were seldom bitten by them while they were under observation. The fly is conspicuous not only by its size but by the formidable sound which it makes, and before it settled on the skin the natives were generally prepared for it and dealt with it immediately.

*Development of ovaries in Simulium fed on human blood.*

The condition found at the time of capture is shown in fig. 4, the ovaries appearing as purely abdominal organs of fusiform shape with small circular ova with a more dense area like a nucleus. When the specimens captured had taken no blood or only a small amount, the ovaries did not undergo development during the next few days.

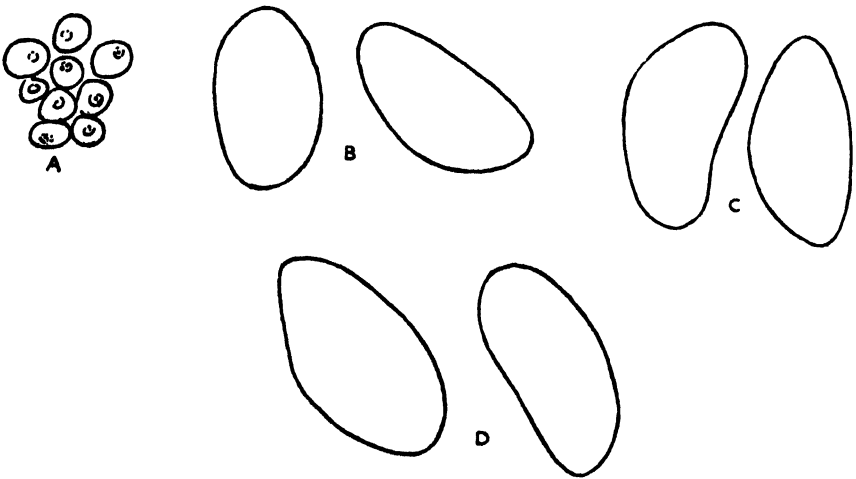


FIG. 4. Ova of *S. damnosum*. A.—Unfed; B.—Fourth day after feed; C.—Fifth day, D.—Seventh day.

\* The specimens belong to a new species which will shortly be described by Major E. E. Austen in the *Bulletin of Entomological Research*.

In flies which had fed well the ovarian development was rapid and progressive. The appearance of the ova at various stages is shown in fig. 4. By the fourth or fifth day the ovaries had become partly thoracic in position, so that in separating the thorax from the abdomen a portion of the ovaries and their contained eggs was frequently found in the thoracic region. The increase in size of the ovaries proceeded proportionately to the decrease of blood in the gut so that after a week a fly which had fully fed still had a fully-fed appearance, with this difference, that instead of a globular bulge on each side of the abdominal region, there was a tapering enlargement which diminished as it extended backwards from the thorax to the posterior end of the abdomen.

### ACCOUNT OF STAGES

#### (1) *In subcutaneous nodules.*

The fluid obtained by aspiration of nodules with a syringe contained free larvae, and in several instances where the uterus had been punctured, eggs with embryos in them.

##### (a) *The egg.*

The egg measured from  $46\mu$  to  $61\mu$  in length, by  $33\mu$  to  $51\mu$  in breadth; the egg membrane remained practically unstained when dried, fixed in alcohol and stained with hot haemalum.

##### (b) *The embryo in the egg.*

These measured from  $264\mu$  to  $290\mu$  in length, by from  $7\mu$  to  $9\mu$  in breadth; the cephalic clear area measured from  $6\mu$  to  $9\mu$ , the caudal clear area from  $12\mu$  to  $16\mu$ , and the first break from the cephalic extremity was situated at from 21 to 25 per cent. of the length. The nuclei were arranged irregularly in a somewhat scattered manner in the body and were stained to a depth comparable with one group of free larvae in the fluid, but less deeply than another group; these two groups of larvae occurring in the nodules are referred to below in dealing with the free larvae.

##### (c) *The free larvae.*

The larvae which were found free in the nodule fluid presented two types. The first was a large form very similar, in the scattered

arrangement of the nuclei and in staining reaction, to the form found in the egg; the measurements were, as a rule, slightly greater than those of the egg forms. The second was a small form having a more compact arrangement of the nuclei and taking the stain more deeply than either the large larvae or the egg forms. Although there was some overlapping in measurements and in the density of staining in individual forms, the differences of the two types were as a rule easily observable.

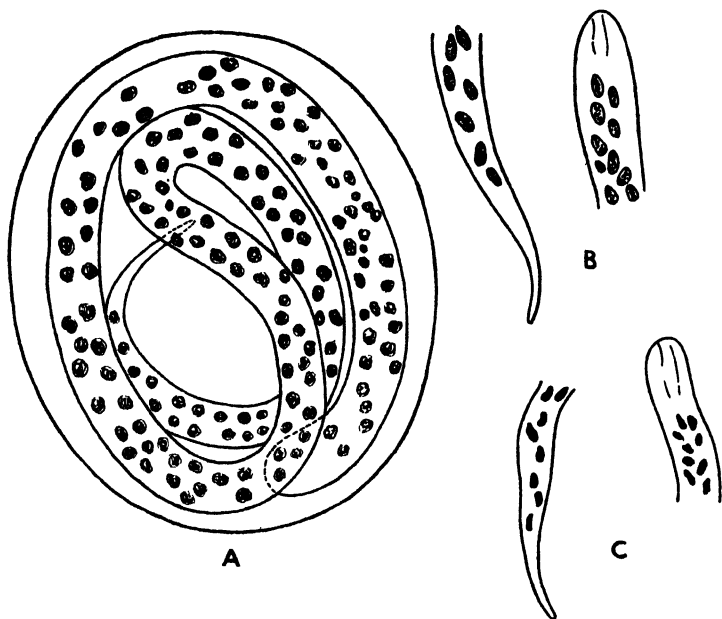


FIG. 5. In puncture fluid from nodule. A.—Larva in egg membrane; B.—Large pale forms of larva; C.—Small deeply stained form.

### *Large forms.*

These varied in length from  $295\mu$  to  $358\mu$ , and in breadth from  $6\mu$  to  $9\mu$ ; the cephalic clear area measured from  $7\mu$  to  $11\mu$  and the caudal from  $13\mu$  to  $18\mu$ ; the first break from the cephalic end was at from 21 to 25 per cent. of the length; in these forms, as also in the egg forms, owing to the irregular disposition of the nuclei, even the first break was sometimes difficult to determine with accuracy.

*Small forms.*

These forms usually measured from  $221\mu$  to  $287\mu$  in length by from  $5\mu$  to  $7\mu$  in breadth; the cephalic clear area measured from  $5\mu$  to  $8\mu$ , the caudal clear area from  $10\mu$  to  $16\mu$ ; the first break was situated at from 22 to 25 per cent. of the length.

(2) *In the skin.*

Great variation in size was evident in the forms derived from the skin. Whereas forms which were measured in fresh preparation varied from  $306\mu$  to  $322\mu$  in length by from  $6\mu$  to  $9\mu$  in breadth, specimens in water, which had been allowed to dry on the slide, and then fixed by absolute alcohol, and stained, showed remarkable differences in dimensions. The majority were found to measure from  $250\mu$  to  $300\mu$  in length; in one case, however, one specimen was found of only  $184\mu$  in length and several other specimens in the

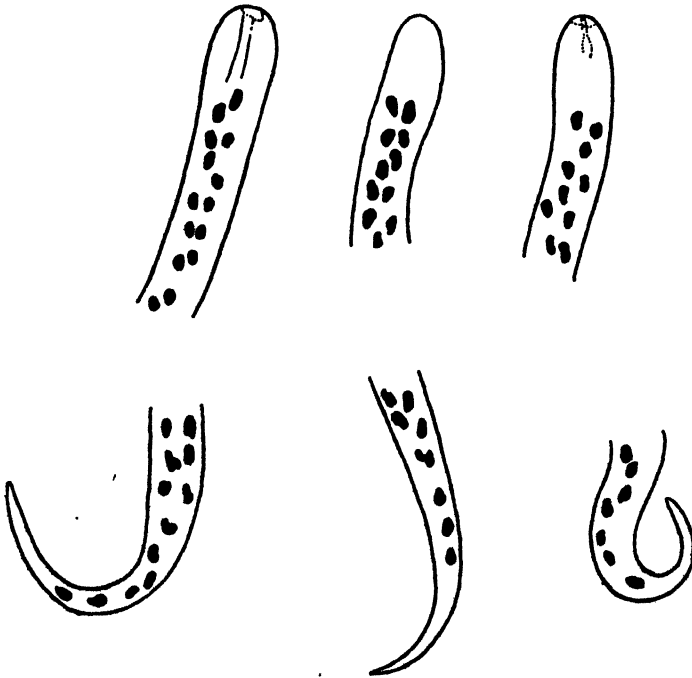


FIG. 6. *O. volutus* forms in gut of *S. damnosum*.

neighbourhood of  $200\mu$ . The larvae in this case were dissected out in water, allowed to dry on the slide, fixed in absolute alcohol and stained by prolonged immersion in dilute carbol methylene blue followed by heated haemalum. In ten specimens measured in this case, the length varied from  $184\mu$  to  $229\mu$ ; the cephalic clear area measured from  $5\mu$  to  $9\mu$  and the caudal from  $9\mu$  to  $21\mu$ ; the first break from the cephalic end occurred at from 20 to 23 per cent. of the length.

The usual breadth of the skin larvae was from  $5\mu$  to  $9\mu$ , the cephalic clear area measured from  $5\mu$  to  $13\mu$ , the caudal from  $9\mu$  to  $21\mu$ ; the first break from the cephalic end was situated at from 21 to 26 per cent. of the length.

(3) *In the gut of Simulium damnosum.*

The forms found in the gut of the fly measured from  $200\mu$  to  $334\mu$  in length by from  $5\mu$  to  $8\mu$  in breadth; the cephalic clear area measured from  $6\mu$  to  $8\mu$  and the caudal clear area from  $10\mu$  to  $17\mu$ ; the first break from the cephalic end occurred at from 20 to 24 per cent. of the entire length. The measurements are set out in the following table.

TABLE XI.

Giving the measurements of the forms so far dealt with.

Form	Length $\mu$	Breadth $\mu$	Clear area		First break at per cent. of length
			Head $\mu$	Tail $\mu$	
1. Nodule :—					
(a) Egg ... ..	46-61	33-51	...	...	...
(b) Larvae in egg ...	264-290	7-9	6-9	12-16	21-25
(c) Free pale forms ...	295-358	6-9	7-11	13-18	22-25
(d) Free dark forms ...	221-287	5-7	5-8	10-16	22-25
2. Larvae in skin ... ..	184-322	5-9	5-13	9-21	20-26
3. Larvae in fly gut ... ..	200-334	5-8	6-8	8-17	20-24

### *Movements of larvae.*

Within the egg in the nodule fluid only sluggish and partial movements of the larvae were observed. The larvae free in the fluid moved actively but the movement was confined to a coiling and twisting movement without progress across the field. The larvae from the skin when freed in a drop of water on a slide were, as a rule, more sluggish in their movements than the free larvae in the nodule fluid. The chief exceptions to this were the larvae obtained in skin sections from the loin in the case of two adult females; the larvae from these cases showed more active movement than did those from the skin or the subcutaneous nodules in the males examined. In the gut of the fly the larvae proved considerably more active in their movements than in either the nodules or the skin; they exhibited even a certain degree of translatory movement when they were in contact with the blood and débris on the slide.

## DEVELOPMENTAL FORMS FOUND IN THE THORAX AND HEAD OF *SIMULIUM*

### *A. In casual flies.*

Certain flies dissected soon after capture contained forms of developing larvae in the thorax and head. The developing larval forms were of various sizes and comprised two very distinct stages, with several intermediate forms.

The two stages were:—(1) Forms of a shape corresponding to those given in fig. 7, with a wide body and definite tail, with or without clear spots in the anterior and posterior portions of the body, and with a more or less developed intestine. A series of such forms ranging in length from  $166\mu$  to  $425\mu$ , and in breadth from  $18\mu$  to  $43\mu$ , was collected from the dissection of the thorax of casual flies. (2) Forms usually of a greater length than those and devoid of definite tail. These forms were, as a rule, considerably narrower than the preceding and differed in having a definite intestine and a slit-like anus; a series of such forms collected from the dissection of the thorax of casual flies gave a range of length from  $560\mu$  to  $660\mu$ , and of breadth from  $18\mu$  to  $28\mu$ .

Developing forms were not often present in large numbers in those casual flies which were infected; in most cases little movement

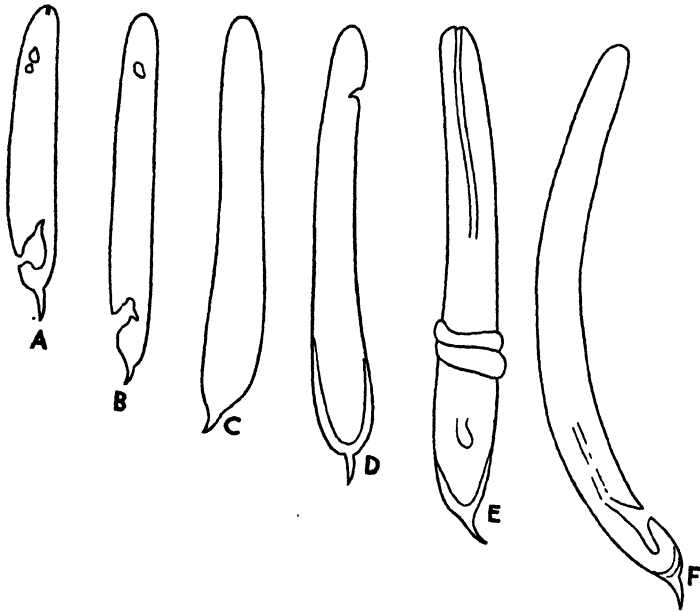


FIG. 7. *O. volvulus* forms with caudal appendage from thorax of wild *Simulium damnosum*. A.— $214\mu \times 34\mu$ ; F.— $425\mu \times 38\mu$ . Forms D, E, and F in process of ecdysis.

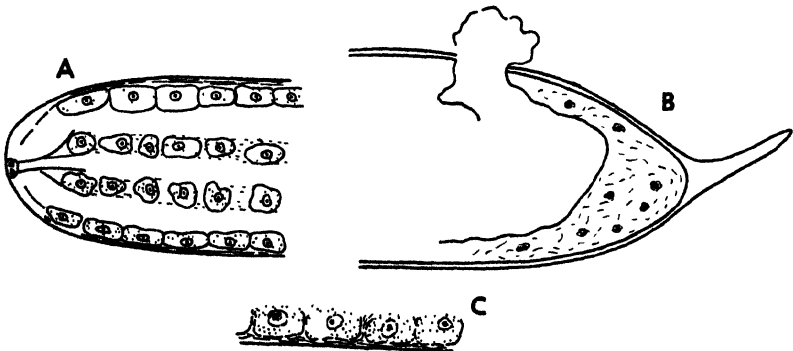


FIG. 7A. *O. volvulus* forms in thorax, wild *Simulium damnosum*,  $266\mu \times 33\mu$ . A.—Head end; B.—Tail end; C.—Subcuticular cell layer, mid-third of body.



was observed beyond a slight flexion of one or other extremity. Some of the large forms, however, continued to move for a short time after liberation from the thorax. The only form which exhibited very active and prolonged movement came out of the thorax attached to the head on separation of the head from the thorax. It moved very actively and incessantly for a period of three hours, showing very considerable power of penetrating masses of débris on the slide. Unfortunately it was lost in the manipulation of the coverslip. This individual is not included in the measurements given.

*B. In flies kept alive after feeding on infected skin.*

The forms found in these flies at various dates after being captured feeding on infected human skin were similar in measurements and in morphology to those found in the flies of group A. A series of wide forms possessing a tail varied in length from  $173\mu$  to  $461\mu$ , and in width from  $17\mu$  to  $45\mu$ ; a series of forms without a tail measured from  $349\mu$  to  $628\mu$  in length, and from  $20\mu$  to  $38\mu$  in width.

Some of the longer forms exhibited active movements, but it was observed that some forms which were active were of smaller size than other inactive forms in the thorax of the same fly. This possibly has a connection with the process of ecdysis.

The numbers of forms dissected out of the thorax in several of the flies which had thus fed on known infected skin was large. In a fly which was dissected sixty hours after thus feeding, no less than 236 developmental forms were recovered from the thorax. In another fly which died on the fifth day sixty forms were found. In a fly which lived until the seventh day ten forms of a much more advanced stage of development were found. It was observed that in some flies there were present forms of different stages. The explanation which naturally suggests itself is that those flies had acquired infection at some period previous to, as well as at the time of, their feeding on the infected case. Such an explanation will not, however, suffice to account for a case such as the following. The flies referred to above which had fed sixty hours previously on infected skin presented, in addition to the early thoracic developing forms, a form which appeared to be at the same stage of development

as the forms just ingested by the fly, and a form of shorter and broader type evidently about midway in development. It was in excellent condition and stained well. The explanation of this probably is either that some of the forms ingested by the fly have not developed sufficiently before their ingestion to enable them to keep pace with the rest, or that they have for some reason failed to reach a suitable portion in which further development can proceed.

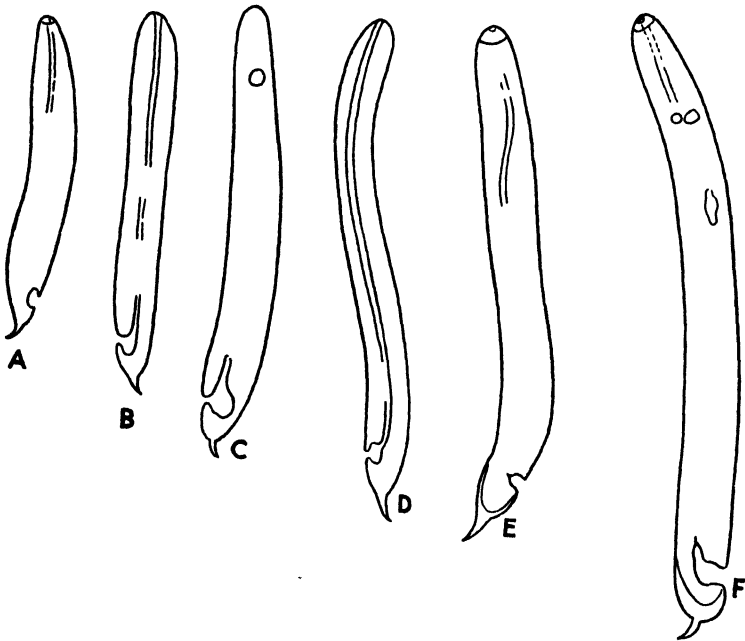


FIG. 8. *O. volutus* forms with caudal appendage from thorax of *S. damnosum*, five days after feeding on infected skin. A.— $223\mu \times 36\mu$ ; F.— $440\mu \times 36\mu$ . Forms E and F in process of ecdysis.

The argument which is applicable to this case can probably, with justification, be applied to cases at a later date where, in the same fly presumably infected at the time of feeding on known infected skin, there appear, in addition to forms which represent a correct stage of development for the period as judged by the average development in other flies, forms which represent an earlier stage.

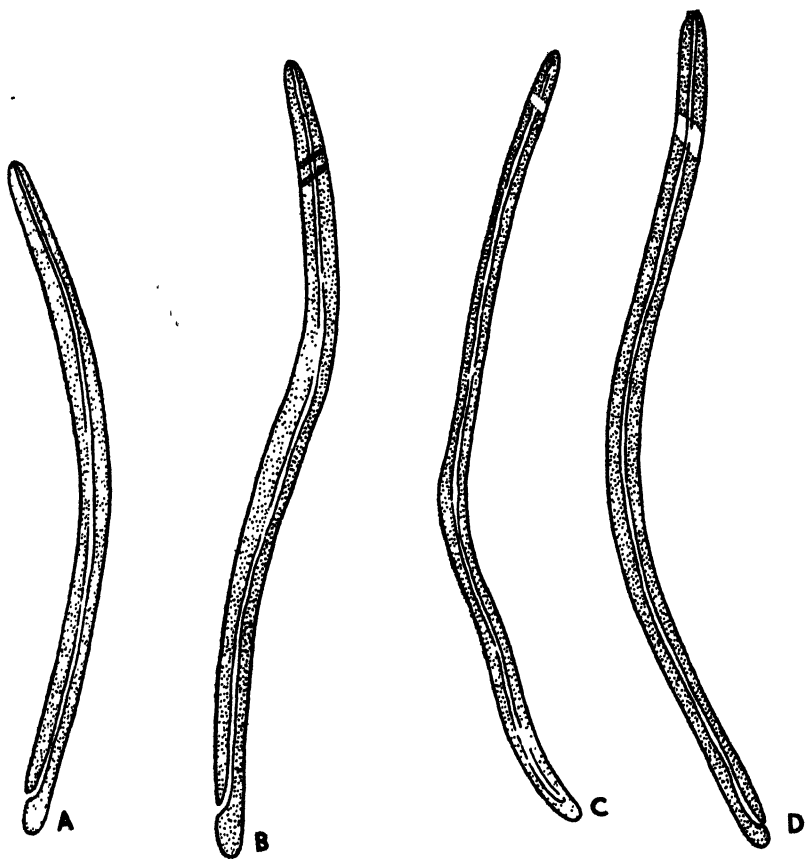


FIG. 9. A.—*O. volvulus* form from thorax of *S. damnosum*, seven days after feed.  $505\mu \times 25\mu$ .  
 B.—*O. volvulus* form from thorax of *S. damnosum*, seven days after feed.  $592\mu \times 32\mu$ .  
 C.—*O. volvulus* form from thorax of *S. damnosum*, wild, seven days after feed.  $616\mu \times 25\mu$ .  
 D.—*O. volvulus* form from thorax of *S. damnosum*, wild, seven days after feed.  $657\mu \times 28\mu$ .

*Ecdysis.*

It is evident that throughout the process of development of the larva several changes are brought about which could most readily be explained on the basis of ecdysis. If this is not the explanation it is difficult to understand how, at various stages, the larva undergoes the considerable variations in length and breadth observed. Thus in the tumour fluid the large pale form which resembles the egg form is associated with a smaller and darker-stained form with compact nuclei; in the skin, only forms corresponding to the latter type were found, but again with a diminution in length of some individuals. In the thorax the shortest form found in any fly measured only

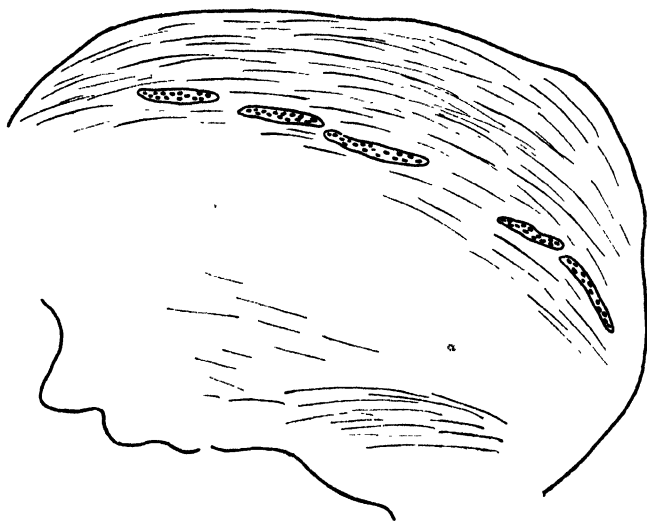


FIG. 10. Section of thorax of *S. damnosum* on fifth day after feeding on infected skin, showing portions of developing forms of *O. volvulus* larvae.

166 $\mu$ , yet in the skin of infected persons the shortest form found was 184 $\mu$ , while in the gut of infected flies the shortest form found was 200 $\mu$ . What seems to be actual ecdysis was only observed in the case of the transition from the tailed thorax form to the thoracic form without tail; it was observed that in some cases the active forms later developed in the thorax were accompanied by forms slightly longer and immobile, and the appearances suggested that at this stage also, an ecdysis had occurred from the more elongated immobile form to the active form.

## SUMMARY

1. *Onchocerca volvulus* infection is common in the Konno District of the Protectorate of Sierra Leone ; larvae of this parasite were found in the skin of 45 per cent. of persons examined systematically.

2. A definite relationship between diseases of the skin or diseases of the eyes and the infection with *O. volvulus* as judged by the presence of larvae in the skin could not be established.

3. *Simulium damnosum* is very prevalent in the hilly country, which is covered with bush and grass, and has numerous streams and rivers ; the lesion inflicted on the skin by this species in biting is such as would dislodge the larvae of *O. volvulus* in the skin.

4. Dissection of wild insects showed :—

(a) In 780 dissections of the gut an infection of 2.6 per cent. with larvae morphologically identical with those of *O. volvulus*.

(b) In 1,320 dissections of the thorax a larval infection of over 1 per cent.

5. By allowing wild flies to feed on restricted and heavily-infected areas of the skin the gut infection was raised to 80 per cent. in one experiment and the thorax infection to nearly 82 per cent. in another experiment ; the developing forms of *O. volvulus* were found in the thorax after the infecting feed up to the seventh, eighth, and tenth days, after which period no insect survived.

PLATE I

## EXPLANATION OF PLATE I

Fig. 1. Nodules on both trochanters.

Fig. 2. Nodules on ribs (1), and trochanter (2). Puncture positive in both.

Fig. 3. Painful nodules and a much wrinkled skin.

Fig. 4. Case 59. Nodules on right elbow (1), and right trochanter (2).



1.



2.



3.



4.





## PLATE II

## EXPLANATION OF PLATE II

- Fig. 1. Case 59. Nodules on left elbow (1), left trochanter (2), and left knee (1).
- Fig. 2. Case 59. Nodules (near view, right side) on elbow (1), and trochanter (2.)
- Fig. 3. Capturing *S. damnosum*.
- Fig. 4. Type of country infested by *S. damnosum*.



1.



2.



3.



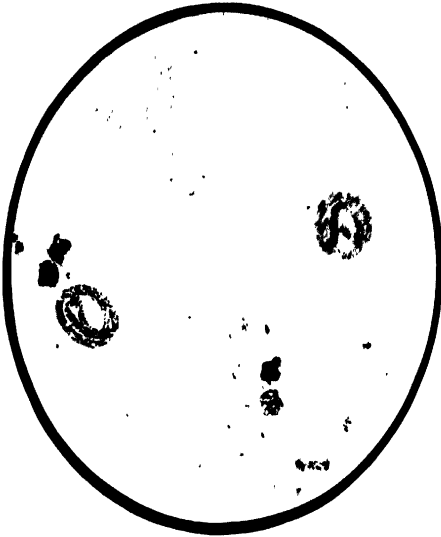
4.



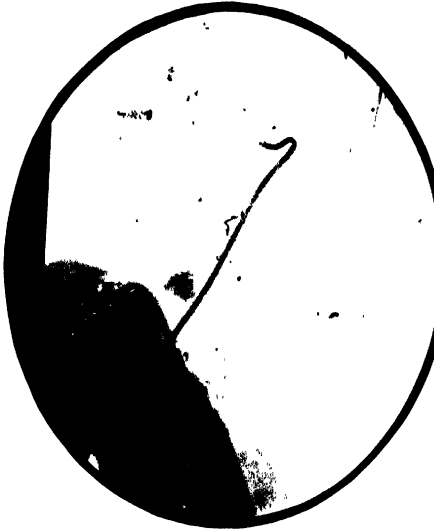
PLATE III

## EXPLANATION OF PLATE III.

- Fig. 1. Micro-photograph showing two eggs of *O. volvulus* in puncture fluid from a subcutaneous nodule.  $\times$  about 200.
- Fig. 2. Micro-photograph of larva of *O. volvulus* in puncture fluid from a subcutaneous nodule.  $\times$  200.
- Fig. 3. Micro-photograph of two larvae of *O. volvulus* from skin.  $\times$  200.
- Fig. 4. Micro-photograph of larvae of *O. volvulus* from gut of *S. damnosum*.  $\times$  200.



1.



2.



3.



4.

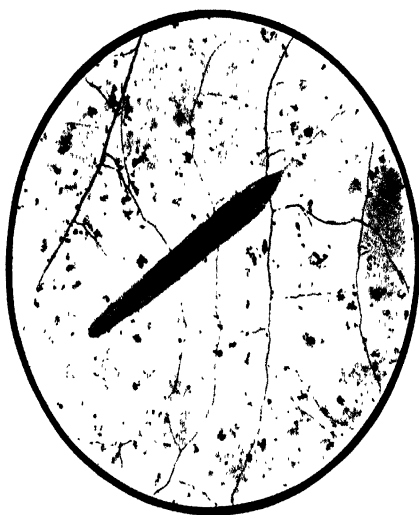




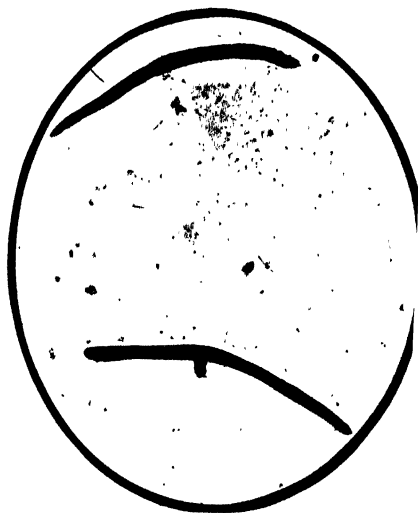
**PLATE IV**

## EXPLANATION OF PLATE IV

- Fig. 1. Micro-photograph of larva of *O. volvulus* from thorax of *S. damnosum* sixty hours after ingestion.  $\times 200$ .
- Fig. 2. Micro-photograph of two larvae of *O. volvulus* from thorax of *S. damnosum* seven days after ingestion.  $\times 125$ .
- Fig. 3. Micro-photograph of section of skin showing larvae of *O. volvulus*.  $\times 200$ .
- Fig. 4. Micro-photograph showing skin lesion produced by the bite of *S. damnosum*.  $\times 20$ .



1.



2



3.



4



# OBSERVATIONS ON THE DEVELOPMENT OF HOOKWORM LARVAE

## PART I

BY

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(Received for publication 4 November, 1925)

Neish (1913), in Jamaica, stated that ova and Ancylostome larvae were destroyed by normal saline, and on these grounds he explained the absence of hookworm infection on two estates in Portland, where the labourers' barracks were situated on the sea shore. Caldwell (1922), working in Honduras, suggests that the relatively low infection rate among the inhabitants of villages on the shore compared with that found in villages further inland is due to the lethal action of sea water on hookworm larvae. On the other hand, Nicoll (1917) found that even with a 6 per cent. solution of salt, several eggs were capable of hatching after as long as six or seven days in the salt. Nicoll's results suggest that it is something besides the mere presence of salt which prevents the development of hookworm eggs and larvae, and the following series of experiments were undertaken with a view to finding out, if possible, what was the real reason underlying the observations of Neish and Caldwell.

As Maplestone (1924) has shown, *Necator americanus* is much commoner than *Ancylostoma duodenale* in Freetown, and the following results should be taken as applying to the former species. Artificial cultures were used throughout, for although the soil in and around Freetown must be heavily infected with the larvae of *N. americanus*, the results would be unreliable if naturally infected soil were used, because the numerous cats and dogs present being heavily infected with *A. caninum* and *A. ceylanicum*, these larvae would be found in large numbers as well, and Stekhoven and Stekhoven-Meyer (1924) have shown that the larvae of these two species are indistinguishable from those of the worms infecting human beings. In this connection,

the writer is not aware that *A. caninum* and *A. ceylanicum* are present in the Islands of Trinidad and Porto Rico, but it is a remarkable fact that in the numerous papers of the series 'Investigations on the Control of Hookworm Disease,' recently published by Cort and many other American workers in these places, the possibility of confusing the larvae of *N. americanus* with those of the above species is not referred to.

### TECHNIQUE

All the work was done in the laboratory at room temperature. The cultures were placed on a bench beneath large windows and were covered with glass bell jars; they thus received plenty of light but were never in direct sunlight.

Except in a few special cases cultures were always examined on the seventh day of growth, so that comparison between them would be as accurate as possible.

The materials used in the cultures were powdered wood charcoal, laterite, which is the natural soil in Freetown, and sand taken from the sea shore below high-tide level. The laterite and sand were heated in enamel saucepans to between 60 and 70 degrees Centigrade for about twenty minutes. This temperature did not dry or char the material and was always found sufficient to destroy all nematodes, whether larvae or adults, that were in the soil. They were then passed through a sieve with a mesh of 4 mm., to remove the larger stones. The materials were stored in tins, and in a few days the laterite became perfectly dry, whereas the sand remained moist for months. The sand was found to be slightly alkaline (pH 7.6) and the laterite was neutral in reaction. The relative weights of charcoal, laterite and sand were 2, 3, and 9, respectively per volume, therefore quantities for making cultures were measured by volume and not by weight, so that all cultures were of equal size and the mixture of faeces and culture medium were in the same proportions on all occasions. The amount of faeces used in a culture was always 3 grams. It was soon found that charcoal mixed with laterite was no better than plain laterite as a culture medium, so that the charcoal was discontinued.

Wire gauze with a mesh of 1.158 mm. was made into small

baskets measuring 4 cm. square and 2 cm. in depth, thus having a capacity of 32 c.c. Small cultures, consisting as they did of 25 c.c. of earth and 3 grams of faeces, easily fitted into these baskets. For isolation of larvae from the cultures the baskets, as a whole, were placed in the isolation apparatus. The advantages of this method were that handling of cultures was simple and rapid, and there was no danger of loss of any of the culture by transferring it to a fresh sieve; the wire gauze also allowed water to drain away freely, so the cultures were never standing in excess water, being unduly water-logged in consequence. For some cultures on a larger scale that it was found advisable to make, wooden boxes in the shape of cubes, 10 cm.  $\times$  10 cm., were made.

For isolation of larvae from cultures, funnels of suitable size for the culture concerned were fitted with a short piece of rubber tubing closed by a spring clip, in every way similar to the apparatus used by Cort *et al.* (1922). The funnels were lined with a double layer of plain white muslin as it was found that a single layer let too much soil through. The method of dealing with the small cultures has already been described; for the large cultures wire gauze sieves of suitable size were made, using gauze of the same mesh as that from which the small baskets were made. The cultures in the latter case had to be transferred from the boxes to the sieves. After being placed in a suitably sized funnel and the spring clip being applied to the rubber tubing, water at a temperature of 50° C. was poured in until the soil was partly submerged. By starting with water at 50° C. it was reduced to about 45° C. by the time it had come in contact with the culture; this temperature has been shown by Cort *et al.* (1922) to be the optimum for the isolation of larvae, and a few preliminary experiments confirmed this. The water was poured carefully down the side of the funnel, avoiding contact with the culture, until it rose sufficiently high to reach the soil from below. A series of cultures was always put up for isolation in the afternoon and examined on the following morning, that is, fifteen to eighteen hours afterwards.

Many cultures produced thousands of larvae, which rendered counting all of them impossible, so the following method was adopted. Centrifuge tubes holding about 15 c.c. were marked at 10 c.c. and 5 c.c. The clip at the end of the rubber tube was slowly



released, allowing exactly 10 c.c. of water from the culture to flow into the tube. This amount of water was found to contain all the larvae that had come out of the culture. The tube was then centrifuged to throw all the larvae to the bottom and then 5 c.c. of water were drawn off, leaving all the larvae from a given culture in 5 c.c. of water. A Wassermann pipette, cut short to facilitate handling, was fitted with a rubber teat, and this was used for collecting samples for counting. An even suspension of the larvae was first made by drawing up and forcibly expelling portions of the contents of the tube; using the pipette for the purpose, it could easily be determined by the naked eye when this had taken place. The pipette was now filled and 0.1 c.c. allowed to run on to a slide. This operation was repeated five times and five slides from each culture were prepared; but after the taking of each 0.1 c.c. sample, care was taken to mix the suspension of larvae thoroughly, because they were found to settle to the bottom very rapidly. The number of larvae in each of the five drops was counted, the five totals added, and this figure being multiplied by ten gave the number of larvae in the original 5 c.c. of water, that is, the number of larvae isolated from the culture. A cover slip was found unnecessary as the larvae could be counted easily with a low power (Zeiss objective A, and eyepiece 4). In counting, a mechanical stage was used and the whole drop of water passed under review; to avoid counting larvae more than once or missing them altogether, those larvae that only partly showed in the lower edge of the field were counted and those that only partly showed in the upper edge of the field were omitted. A drop of Lugol's solution was added before counting because it rendered the larvae motionless at once; this was found necessary for, with a large number of larvae wriggling actively about, it is not possible to count them accurately, and a further possible source of error is that some larvae may pass out of the field either before or after being counted, and the same larvae might thus be encountered in another part of the drop. Another advantage of the use of Lugol's solution is that it enabled the distinction between hookworm and *Strongyloides* larvae to be made rapidly and accurately; this is a simple matter in the ordinary way, but when there are large numbers of larvae wriggling about and some of the hookworm larvae have lost their sheaths, it is not so easy to distinguish between them.

In the following experiments it will be noted that the numbers of larvae isolated in different series of cultures are very different ; this is because faeces from many individuals were used during the course of the work.

For the sake of brevity all the experiments given below are expressed as a single figure which, however, represents the average of a large number of individual experiments.

SECTION I. The Effect of Sea Water and Sea Sand on the Development of Hookworm Eggs and Larvae.

#### EXPERIMENT 1.

Portions of faeces 3 grams in weight were mixed with laterite and charcoal, half of the cultures were kept moist with sea water, and half with tap water as controls.

Cultures moistened with sea water produced 313 larvae per gram of faeces, and the controls moistened with tap water produced 328 larvae per gram of faeces.

This clearly shows that sea water alone has no effect on the development of hookworm eggs and larvae.

#### EXPERIMENT 2.

The effect of sea sand was next tried, being controlled by cultures of faeces in charcoal and laterite mixture, and all cultures were moistened with tap water. Sand and laterite prepared in the manner described were used throughout. In a series of over twenty different experiments on these lines, using faeces from several individuals, the number of larvae per gram of faeces recovered from sand cultures varied from nil to about 20 per cent. of the numbers recovered from the charcoal-laterite controls.

Next, a series of seven cultures in sand and a similar number of controls in charcoal-laterite mixture were put up. One each of these cultures was examined every day from the first to the seventh day of growth, and it was found that on the first and second days the sand cultures and the controls produced an approximately equal number of larvae ; but from the third day onwards the numbers in the sand cultures gradually diminished, whilst the controls remained unaltered. This clearly indicated that, whatever the action of the sand might be, it was exerted on the young larvae and not on the eggs before hatching.

A portion of previously heated sea sand was washed by allowing tap water to percolate through it for twenty-four hours. It was then dried and used as a culture medium, controlled by cultures in charcoal-laterite and unwashed sea sand. In addition to mixing with sand alone, various modifications such as mixing faeces with sand-charcoal-laterite in various proportions or covering cultures with a layer of one or other of the media, were also tried. Three grams of faeces were used in all cases, the cultures were grown for seven days, and the results are expressed in terms of the number of larvae isolated from one gram of faeces. In the following series of experiments unwashed sea sand is called 'sea sand,' washed sea sand is called 'washed sand,' and charcoal-laterite mixture is called 'earth.'

## EXPERIMENT 2a.

Faeces-earth-washed sand in equal parts produced	...	...	...	400 larvae.
Faeces-earth-sea sand in equal parts produced	...	...	...	1,440 larvae.
Faeces-earth, covered with an equal volume of washed sand produced	...	...	...	530 larvae.
Faeces-earth, covered with an equal volume of sea sand produced	...	...	...	90 larvae.

## EXPERIMENT 2b.

Faeces-washed sand produced	...	...	...	...	...	143 larvae.
Faeces-sea sand produced	...	...	...	...	...	27 larvae.

## EXPERIMENT 2c.

Faeces-earth not covered produced	...	...	...	...	295 larvae.
Faeces-earth covered with washed sand produced	...	...	...	...	108 larvae.
Faeces-earth covered with sea sand produced	...	...	...	...	79 larvae.
Faeces-washed sand not covered produced	...	...	...	...	102 larvae.
Faeces-sea sand not covered produced	...	...	...	...	10 larvae.

## EXPERIMENT 2d.

Faeces-earth not covered produced	...	...	...	...	920 larvae.
Faeces-earth covered with an equal volume of same produced	...	...	...	...	957 larvae.
Faeces-earth covered with an equal volume of washed sand produced	...	...	...	...	727 larvae.
Faeces-earth covered with an equal volume of sea sand produced	...	...	...	...	100 larvae.

## EXPERIMENT 2e.

Faeces-earth produced	...	...	...	...	...	860 larvae.
Faeces-earth (1 part)-sea sand (1 part) produced	...	...	...	...	...	27 larvae.
Faeces-earth (1 part)-sea sand (2 parts) produced	...	...	...	...	...	37 larvae.
Faeces-sea sand produced	...	...	...	...	...	0 larvae.

The above series of experiments indicates that sea sand has a lethal effect on hookworm larvae when used under the conditions of these experiments, and they show that unwashed sand is more powerful than washed sand. It was now decided to test the same materials, using the same amount of faeces but much larger amounts of earth and sand, and for this purpose wooden boxes 10 cm. square by 10 cm. in depth were employed in place of the small wire gauze baskets. The boxes were filled with laterite to within one inch of the top, the faeces was placed on the surface and covered with a thin layer of the material to be tested. From now on charcoal-laterite mixtures were replaced by plain laterite as it was found that the latter was a slightly more favourable culture medium.

#### EXPERIMENT 2f.

Faeces-earth covered with laterite half-inch and moistened daily									
produced	...	...	...	...	...	...	...	...	446 larvae.
Faeces-earth covered with sea sand half-inch and not moistened									
produced	...	...	...	...	...	...	...	...	483 larvae.
Faeces-earth covered with sea sand half-inch and moistened daily									
produced	...	...	...	...	...	...	...	...	518 larvae.

This series of experiments indicates that sea sand seems to be effective only in small cultures and not in large ones ; this is difficult to understand as, with a much greater proportion of sand to faeces, it might be expected that the effect would be more marked in large cultures than in small ones. As a check on this contradictory result a fresh series of small cultures was made in the wire baskets.

#### EXPERIMENT 2g.

Faeces-earth covered with earth produced	...	...	...	...	382 larvae.
Faeces-earth covered with sea sand produced	...	...	...	...	346 larvae.

This apparently showed that the sand had lost its former effect, and as the sand used up to this time had been stored in the laboratory for some months it was thought this might be the reason. Accordingly a fresh lot of sand was procured and after being heated in the same way as the first lot it was tried under identical conditions.

#### EXPERIMENT 2h.

Faeces-earth covered with earth produced	...	...	...	...	5,612 larvae.
Faeces-earth covered with old sand produced	...	...	...	...	5,042 larvae.
Faeces-earth covered with new sand produced	...	...	...	...	3,050 larvae.

This showed that the new sand was more effective than the old sand, but it was not nearly so powerful as the old was when first collected.

## EXPERIMENT 2j.

Testing the new sand in larger quantities, viz., in boxes.

Faeces-earth covered with earth produced	...	...	...	1,327 larvae.
Faeces-earth covered with new sand produced	...	...	...	933 larvae.

Reduction of the results of the two experiments 2h and 2j, to the same proportion, gives 1.5 : 1.47, which shows that the effect of the new sand is practically the same in small and large cultures. But in less than a week it was found that the new sand was as ineffective as the old.

All the experiments hitherto recorded have been subjected to the same conditions of moisture except where the contrary is stated, and the cultures in any given series have always been wetted with equal amounts of water and at the same time.

The foregoing experiments were carried out from August to the beginning of November, at which time the sand was found to have become ineffective. The reason for the loss of power in the sand was puzzling until it was realised that the change in its efficacy coincided with the change from the wet to the dry season. During the rains in Sierra Leone, humidity is high and evaporation is, consequently, slight. It had been noted that sand cultures were always more sodden with water than corresponding earth cultures, even when equal amounts of water were used for moistening them, suggesting that earth is able to absorb more water than sand. The idea, therefore, suggested itself that the suspected lethal effect was not in the sand *per se* but was due to the inability of the sand to absorb water as readily as earth, with consequent excess of water in the former type of cultures. This excess was more marked in the wet than in the dry seasons for, during the latter, the greater evaporation would allow the sand cultures to approximate more nearly to the earth cultures with respect to the amount of free water present. This hypothesis was supported by the observation that, in boxes which contained a large quantity of earth below the cultures, the sand was not effective at all, and this could be explained as follows :—The large quantity of absorbent earth beneath the sand drained excess of water out of the latter, thus allowing the sand to become a suitable culture medium.

The next series of experiments was devised with the object of testing the accuracy of the above theory. Two cultures were put up

in sand and two in laterite, one of each being used as controls. In this case the controls had only enough water added to keep them just moist, the sand control requiring much less water than the laterite control. The other cultures were freely moistened daily so that they were always in a state of saturation. This experiment was repeated six times and the results are expressed as an average of the whole.

#### EXPERIMENT 3a.

Faeces-laterite kept just moist produced	...	...	...	...	303 larvae.
Faeces-sand kept just moist produced	...	...	...	...	300 larvae.
Faeces-laterite freely moistened produced	...	...	...	...	117 larvae.
Faeces-sand freely moistened produced	...	...	...	...	120 larvae.

This clearly shows that sea sand and earth, when under equal conditions of moisture, are of the same value as culture media, and as a corollary of this it is evident that, when moistened with equal quantities of water, sand appears a worse medium than laterite because of its inability to absorb water as readily as the latter. Further confirmation of this was obtained by the following series of experiments. They were devised with the object of obtaining as nearly as possible the condition of faeces lying on the ground in the water-logged soil at the edge of a stream or pond.

#### EXPERIMENT 3b.

Small glass jars about 15 cm. in height and 3 cm. in diameter were filled with laterite to within about 4 cm. of the top. Portions of faeces, 3 grams in weight, were mixed with 25 c.c. of laterite and placed on top of the laterite in the jars. The surface of the culture was not flat but was sloped upwards to one side of the jar, so as to reach nearly to the top. Water was now poured into the jar until it just reached the junction of the original soil and the culture, and it was kept at this level continuously by making good every day any loss from evaporation. The effect of this was that the cultures were always completely saturated with water but were not immersed in it. A similar set of jars was prepared, using sea sand instead of laterite, and sea water instead of tap water. For examination the cultures were carefully removed from the surface of the original soil in the jars and were placed in wire baskets for isolation in the usual way. The cultures were then drained off, allowed to remain in the baskets for another week, being kept just moist meanwhile, and

they were then tested by isolation a second time. Two series of cultures were prepared, one being kept in the jars for a week and the other for two weeks before isolation.

SERIES 1 (kept for seven days).

Laterite culture produced...	...	...	...	50 larvae.	} 1st isolation.
Sand culture produced ...	...	...	...	7 larvae.	
Laterite culture produced...	...	...	...	67 larvae.	} 2nd isolation.
Sand culture produced ...	...	...	...	197 larvae.	

SERIES 2 (kept for fourteen days).

Laterite culture produced...	...	...	...	230 larvae.	} 1st isolation.
Sand culture produced ...	...	...	...	20 larvae.	
Laterite culture produced...	...	...	...	0 larvae.	} 2nd isolation.
Sand culture produced ...	...	...	...	223 larvae.	

Control cultures in gauze baskets produced 820 larvae, and as the residual soil in the jars did not contain any larvae it is clear that saturation of soil containing faeces is sufficient to reduce greatly the number of larvae that will develop from it even if brought into more favourable surroundings later on. Payne (1922) has noted the fact that the death rate of hookworm larvae is high in water-logged soil, and the foregoing experiments seem to be a clear confirmation of this.

## CONCLUSION

The evidence of the foregoing experiments tends to show that sea water and sea sand *per se* have no effect in preventing the development of hookworm eggs and larvae.

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# BREEDING PLACES OF ANOPHELINE MOSQUITOS IN AND AROUND FREETOWN, SIERRA LEONE

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## PLATE V AND MAP

The survey, details of which are given in the present paper, was undertaken during the period 26 May to 9 September, 1925, that is, at the commencement and during part of the rainy season.

We are indebted to H. O'Hara May, Esq., M.D., Deputy Director of Sanitary Services, and to the officials of the Sanitary Department, for much valuable assistance which they rendered throughout the period of the investigation.

At various times during the last twenty-five years, investigations have been made by different observers as to the prevalence and distribution of mosquitos in Freetown. During this period there have been great and progressive reductions in the number of mosquitos in general. The findings of Ross, Annett and Austen (1900), and Stephens and Christophers (1900), who examined the town and its environs for mosquitos, prove that the anophelines were present twenty-five years ago, in numbers vastly greater than could be found by any subsequent observers. It has, in fact, frequently been recorded by the latter that in Freetown itself, at any rate, mosquitos are by no means plentiful and rarely obtrude themselves in such numbers as to constitute a cause of physical discomfort. Thus Bacot (1915) writes of the apparent absence and actual rarity of mosquitos in Freetown. Blacklock (1921), who made a survey of anopheline breeding places in the town at the end of the dry season, found



anophelines practically entirely confined to their dry season residual breeding places, which were the pools in the courses of the streams which traverse the town and chiefly at the points above and below the area occupied by streets.

The present detailed survey has included not only the town portion but also, in some cases, the hilly portions of these streams far beyond the town boundaries. A number of species of *Anopheles* have been found, which have either not previously been recorded from the neighbourhood of Freetown or have been known only from a few specimens.

A plan of Freetown, issued by the Public Works Department, Sierra Leone, 1911, is reproduced in part, reduced to about two-fifths and slightly altered, at the end of this report.

The following species were found :—\*

1. *Anopheles costalis* Theo.†
2. *A. funestus* Giles.
3. *A. rhodesiensis* Theo.
4. *A. smithii* Theo.
5. *A. marshalli* var. *freetownensis* Evans.
6. *A. squamosus* Theo.
7. *A. theileri* Edw.
8. *A. domicolus* Edw.

#### THE DISTRIBUTION AND BREEDING PLACES OF THE SPECIES OF ANOPHELES FOUND

##### *A. costalis* Theo.

At the beginning of the investigation this species was found breeding in the stream which opens at Magazine Wharf (see Map), in Nicol's Brook, in Sander's Brook and in the Alligator River. The breeding places were not evenly distributed along the courses of these streams; in the first two the most prolific breeding places were in the portions of the stream below the town, extending from the streets to the sea. Through the town there were often long stretches which proved entirely free from larvae, but they were found again in numbers at the outskirts of the town.

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\* All these species were determined from adults bred from the larvae or pupae.

† Christophers (1924) has shewn that the correct name for this species is *Anopheles gambiae* Giles.

There are several points of interest which may be referred to in detail. For example, the stream which opens at Magazine Wharf is, until just before its entrance to the sea, contained in a laterite drain, and here its flow is fairly rapid ; there is little or no vegetation and the water is used for washing clothes at various points. Throughout this portion no larvae of *Anopheles* were present. After it passes the railway, however, the stream bed widens out into a natural bed of a rocky and steep nature ; the pools found here contained abundant larvae, even to within a few yards of the salt water. Again, in Sander's Brook a notable feature was the regular occurrence of the breeding places at those points at which a lateral drain from the streets joined the main stream. In a stretch of stream running through the town are nine such junction drains ; at six of these, larvae were found in the semi-stagnant weedy area which existed near the junction ; they were not found in large numbers in the stream itself at these points. Certain negative findings are worth recording, e.g., no larvae of this species were found in Granville Brook, skirting the east of the town or, during July and August, in the top of Sander's Brook, above Mendi Street, where the water course has been trained so that the stream flows between loose banks of laterite stones. In September, however, larvae were taken at the latter place in the area of overflow caused by the heavy rains. Isolated breeding sites were found away from the streams in the areas adjacent to them, usually in small seepage waters or in laterite drains, especially on the east side near the outskirts of the town.

In addition to breeding places in these natural and semi-natural waters, there were breeding places of this species in such sites as the following :—

1. Pools in laterite street drains.
2. A concrete washing tank (Pl. V, fig. 4).
3. Canoes lying on the beach at various points.
4. Shallow pits dug in laterite and containing clean water or, as in one case, foul water with an accumulation of decomposing vegetable refuse.

Outside the town, towards the hills behind it, this species was only rarely found in tracing the streams to their source in the hills. The upper part of Nicol's Brook outside the town is rocky, steep

and well shaded by trees; here *A. costalis* was replaced by *A. smithii*. It was remarkable to observe the suddenness with which the latter replaced the former species just beyond the town.

Three collections of larvae taken from water lying in exposed, flat stones, in compounds at Hill Station, were sent to us by the Sanitary Department. None of these were larvae of *A. costalis*.

In general, it may be said that in Freetown *A. costalis* breeds most freely in pools at the edges of slowly running portions of the streams; these pools may or may not be isolated from the current. It is often difficult to say whether a pool is actually separated from the current, but it is frequently possible by stirring up the mud or sand to observe that there is a current of water circulating through pools that are apparently isolated from the stream. While vegetation is usually present in sites containing large numbers of larvae, numerous larvae have been found in water apparently free from vegetation. *The breeding places, almost invariably, are so situated as to receive a considerable amount of sunlight.* The bottom of the pool as well as the sides may be mud, rock, sand or gravel. The character of the water varied from perfectly clear to discoloured and, in one case, foul with rotting vegetation.

If the discovery of large numbers of eggs indicates a preferential breeding place, the following observation from Nicol's Brook may be of interest. Three pools of similar character yielded hundreds of eggs; they were small, shallow pools each containing about a gallon of water, situated beside rocks in the bed of the stream. In each case the bottom was gravelly, no vegetation was growing actually in the water but low herbaceous plants overhung it on one side; no shade was present except the small amount afforded by this vegetation and the adjacent rocks.

We did not find this species breeding in small artificial containers such as tins, bottles, native pots, nor in rot-holes in trees, nor in the water contained in the axils of such plants as *Dracaena* (the 'cocked-hat tree').

From the earliest periods of investigation of the anophelines in Freetown it has been evident that *A. costalis* has been the prevailing species breeding in the town. This was the experience of Ross, Annett and Austen (1900), Daniels (1901), Logan Taylor

(1902), and also in more recent times that of Bacot (1915) and Blacklock (1921) ; the last-named wrote ' This (*A. costalis*) appears at the present time to be by far the commonest Anopheline breeding at the end of the dry season in Freetown.' Comparing our findings of to-day (see Map) with those of the earliest observers we may note that while it is clear that there has been an enormous reduction of adults and breeding places since their time, it is equally clear that the numerical predominance of this species still remains. In Freetown, itself, therefore, this important carrier of malaria is the Anopheline, above all others, which has to be reckoned with. It will remain a standing menace certainly until such time as the streams through the town are treated in a radical manner.

*Anopheles funestus* Giles.

This species was only once found by us in the town itself ; we obtained it, however, in two localities adjacent to the town. The breeding site in the town was in Elliott Street in the western area ; four fully-grown larvae being found in water in a small, unshaded pit in laterite into which dead sticks had been thrown ; numerous larvae of *A. costalis* were taken at this spot at the same time and on other occasions. Outside the town one site was in a garden at the point where the road to Kissy crosses Granville Brook ; the larvae were found in a shallow stream of water rapidly trickling among syenite rocks, on dead leaves, with a small amount of vegetation and exposed to the morning sun. The other site was on the portion of Alligator River just above the town (Pl. V, fig. 3) ; they were found in small numbers over a stretch of a hundred yards of river well above the highest breeding place of *A. costalis* found by us on this stream, and separated from it by a stretch of water in which no larvae were found. The larvae at this place were found among weeds growing at the margin of fairly rapidly running water ; the weeds were growing in water that was more than a foot deep in most places ; there was a little tree shade and the sides and bottom of the stream were of soft mud.

It will be observed that these three breeding places were widely different in character.

Ross, Annett and Austen (1900) found *A. funestus* restricted entirely to the eastern part of the town : ' We never found a larva

or an adult of this species west of Government House . . . . Individuals were caught in a house in the town where some cases of fever had occurred . . . . at Leicester, a village high among the hills, and at some other points. The larvae were common in pools in the eastern quarters, at Kissy and other spots.' Daniels, quoted by Ross (1901), noted that *A. funestus* was found near but not in Freetown. Bacot (1915) records *A. funestus* only from outside Freetown at the following places :—Hill Station, Kissy Flats, and the area between Kissy Road and Fourah Bay. Blacklock (1921) also failed to find *A. funestus* in Freetown.

Concerning the breeding places of this species, Logan Taylor (1902) says that it seems to prefer gently running water to breed in, the larvae being found where the currents are sluggish, and especially amongst grass and weeds at the edge. Two of the sites discovered by us agree in most respects with this writer's observations with regard to the character of the breeding places of this species. It would seem that this species has almost disappeared from even the limited area of distribution in the town referred to by Ross, Annett and Austen. To-day, with one exception, it has not proved possible to find breeding places of this species without going outside the inhabited areas of the town. This apparent modification of the distribution may be compared with what may prove to be a similar modification in the distribution of *A. rhodesiensis* which, formerly not recorded from the town itself, has been found by us in scanty numbers in several widely separated spots.

Meantime, it may be said of *A. funestus* that, however important it may prove to be as a carrier of malaria outside the town, it is quite unimportant in Freetown itself when compared with *A. costalis*.

#### *A. rhodesiensis* Theo.

This species was found to occur sparsely over a wide area including a large portion of the town and the surrounding country ; fourteen breeding sites were discovered but the number of larvae found at each site was small. The largest number found was fifteen at one spot (Record No. 205), which was on the outskirts of the town near the Public Works Department. The species was also found on the eastern side of the town as far as Kissy and on the south-western as far as Hill Station, where the larvae were found by

officials of the Sanitary Department, in the water lying in hollows in large flat stones. Although the spot map shows this species to have a wide distribution it must be emphasized that it is extremely rare when compared with *A. costalis*. Its breeding sites and the associated species indicate its adaptability; in the five places in which it was encountered within the town boundary it was always associated with *A. costalis*; in two sites outside the town, in Nicol's Brook, with *A. smithii*, in another in the upper part of Granville Brook, with *A. funestus*, and in still another, at Kissy Flats, with *A. costalis* and *A. squamosus*. Considering the difference in the character of the breeding sites of these associated species, it is obvious that *A. rhodesiensis*, which for some reason is not a numerous species here, is one which is capable of adapting itself to a great variety of breeding places. It was found, for example, beside a stream almost at sea level (Pl. V, fig. 1); at an altitude of 800 feet; in streams; in road-side ditches; in rock-pools; and, finally, in a concrete washing tank (Pl. V, fig. 4). It is not possible to explain at present why this species has not previously been recorded from Freetown, although it has been recorded by Evans (1924) from close to it at the Cape Lighthouse Peninsula, and it is known from other parts of Sierra Leone. It may be that owing to its sparseness it has been overlooked in previous surveys, or that it has a definite and short breeding season; again it may be that it usually fails to develop under laboratory conditions, or finally, that it may be a recent addition to the Anopheline fauna of Freetown. We are not in a position to decide which of these alternative explanations is the correct one. It is not even possible to say whether *A. rhodesiensis* is an efficient carrier of malaria here and at present it would be a matter of some difficulty to obtain sufficient adults for the performance of transmission experiments.

#### *A. smithii* Theo.

This species was only found outside the town in the upper, hilly portion of Nicol's Brook and the Congo River. Thus it is limited to those parts of these streams which are steep, rocky and shaded by overhanging trees.

A detailed survey of this species was made in the first of the two rivers referred to.

Starting from the point just at the boundary of the town where *A. costalis* ceased to be present, *A. smithii* was suddenly found in large numbers. The amount of overlapping of the breeding places of these two species on this stream was limited to the occurrence of isolated specimens of each species in the area of distribution of the other. The stream was traced up Mount Aureole to its sources on Kortright, at a level of over 1,000 feet. The larvae of *A. smithii* were found in large numbers in pools in the rocky bed of the stream. They were mainly concentrated in two areas of the stream, one near the lower limit of their distribution, above the town boundary, and one near the upper limit not far from the source of the stream. In the intervening length of stream they were found in small numbers only.

The general characters of the breeding places of *A. smithii* are as follows. The breeding places (see Pl. V, fig. 2) are on a rocky bottom which is covered as a rule with dead leaves, often with little or no living vegetation; the water in the pools, though apparently still, was usually found to be in direct communication with the current of the stream. A considerable amount of shade was usually present, but the larvae might be exposed to the sun for certain hours daily. Larvae were, however, also found in the margins of still pools where there was abundant vegetation at the edge of the water. In one case larvae were not seen until a tangled mat of roots and creepers, which lay on the rocky bottom of the pool at the side, was lifted up, when numerous larvae were discovered. On two occasions several larvae were found in small pools sunk in the banks of the stream, the water in them almost hidden from view by a tangle of sticks and creepers.

The extreme localisation of this species, which has not hitherto been recorded elsewhere than at Mount Aureole, is of interest. The only records of it so far as we are aware are those of Theobald, who described the species from several female specimens captured on Mount Aureole by Major Smith, R.A.M.C., and, later, referred a female specimen captured by Captain Grattan, R.A.M.C., at Aureole, to this species. Captain Grattan took male specimens of this species and Theobald made them the type species of the genus *Feltinella*, calling them (*A.*) *Feltinella pallidopalpi*; Christophers (1924), in his catalogue of the Anophelines, makes *A. pallidopalpi* a

synonym of *A. smithii*. From the result of breeding out more than a hundred adult specimens from larvae we are able to confirm this synonymy.

It was found that this species continued breeding throughout the wet season, wherever suitable pools occurred in hilly portions of the streams. Searches made at intervals during the period of investigation continued to yield larvae of all stages and also pupae until the investigation ceased on September 9.

*A. marshalli* var. *freetownensis* Evans.

The larvae of this species were found on eight occasions in rock-pools in certain streams outside the town, but were never found in the town itself. Three records were from Nicol's Brook, at and above Aureole Bridge, three from streams near Kissy Bridge, one from a stream flowing to Granville Brook at Cline Town, and one from a tributary of the Congo River at Hill Station. The character of the breeding places may be gathered from the fact that in four cases they were associated with the larvae of *A. smithii*, in one with those of *A. costalis*, and in another with those of *A. funestus*. When found alone the larvae were in streams where a certain amount of shade was present. The larvae were found from just above sea-level to 800 feet above sea-level.

*A. squamosus* Theo.

Larvae of this species were found in one locality only, namely in Kissy Flats, where they were associated with those of *A. costalis*. The site was a shallow unshaded pool in the course of a ditch in laterite, with slowly running water and some vegetation.

*A. theileri* Edw.

Only four records of this species were obtained. Larvae were found once in association with *A. rhodesiensis* and *A. marshalli* var. *freetownensis*, once with larvae of *A. rhodesiensis*, and on two occasions they were not associated with any other species. Three of the records were, however, from places situated far apart; one being situated well outside the eastern, the others outside the southwestern boundary of the town.



*A. domicolus* Edw.

A single specimen reared from a pupa taken in a road-side ditch at Regent, above Freetown, was referred provisionally to this species.

During the course of this investigation material was examined from various natural, small collections of water, contained in tree holes, axils of leaves, and bamboo, pineapple and banana stems. Numerous samples from such sites were examined and in spite of the fact that larvae of some species of mosquito were present in 179 cases, it was remarkable that in no case were the larvae of Anophelines found in such sites. Owing to the closure of wells in Freetown the findings of Allan (1913), who discovered *A. costalis* in twenty-eight of 418 wells, cannot, fortunately, be repeated.

#### SUMMARY OF CHIEF POINTS BROUGHT OUT BY THE SURVEY

Eight species of Anopheles were found either in or near Freetown.

*A. costalis* is still by far the most numerous species in the town itself.

The chief breeding places of *A. costalis* are in and near the courses of the four streams which flow through the town.

During the heavy rains, shallow laterite drains in streets that have no slope are also important sources of larvae. Larvae of this species were always found in situations exposed to a considerable amount of sunlight; they were usually in entirely unshaded places.

*A. funestus* is found rarely in water courses on the eastern and southern sides of the town; it was taken once in the town itself, this being the first record of its presence within the town boundary since 1901.

*A. rhodesiensis* was found to be widely distributed in Freetown and the surrounding hills. It was the species found in situations exposed to the sun at Hill Station.

*A. smithii*. Larvae of this species were very numerous in the pools in the wooded upper part of Nicol's Brook, especially from the middle of May till the end of July. In the height of the rains larvae could only exist in the pools or eddies of the stream sheltered from the force of the current. They were also taken in the Congo River

system just below Hill Station. Considerable tree-shade was usually, but not invariably, present at the breeding places.

*A. marshalli* var. *freetownensis* occurred outside the western, southern and eastern boundaries of Freetown, but not in the town itself. The larvae were almost always found in streams and usually in shaded situations. They were often associated with those of other species.

Other species found were *A. squamosus*, larvae of which were taken in ditches at Kissy, *A. theileri*, larvae of which we found in streams at Hill Station and to the east and south-west of the town, and *A. domicolus* Edw., taken in a ditch at Regent, behind Freetown.

#### KEY TO THE FOURTH STAGE LARVAE OF ANOPHELINES OCCURRING IN SIERRA LEONE.

1. Antenna with branched hair on shaft..... *mauritanus*\*
- Antenna without branched hair on shaft.....2
2. Dorsal plates relatively very large (fig. 4, A)..... *funestus*
- Dorsal plates relatively small and inconspicuous  
(fig. 4, B and D).....3
3. Outer clypeal hairs (fig. 1, A) plumose..... *squamosus*
- Outer clypeal hairs simple or slightly branched  
(fig. 1, B, C).....4
4. Outer clypeal hair more than three-fourths the  
length of the inner (fig. 1, C)..... *smithii*
- Outer clypeal hairs not more than three-fifths the  
length of the inner.....5
5. Thorax without palmate hairs†..... *costalis*
- Thorax with palmate hairs.....6
6. Leaflets of palmate hairs on third to seventh  
abdominal segments without filament (fig. 2, B)...*theileri*
- Leaflets of palmate hairs on third to seventh segments  
with filament (fig. 2, A, C-G).....7
7. Palmate hairs on fifth abdominal segment very large,  
c. 0.2 mm. in diameter (fig. 2, C), sub-median  
thoracic hairs with bases enlarged and fused  
(fig. 6, D)..... *marshalli* var. *freetownensis*
- Palmate hairs on fifth abdominal segment smaller,  
c. 0.15 mm. in diameter (fig. 2, A), sub-median  
thoracic hairs with bases well separated (fig. 6, B)... *rhodesiensis*

\* The larva of *A. obscurus*, Grünb. probably possesses this character.

† Palmate hairs are here understood to be structures having a short stout base from which arise distally a number of leaflets mostly sub-equal in length.

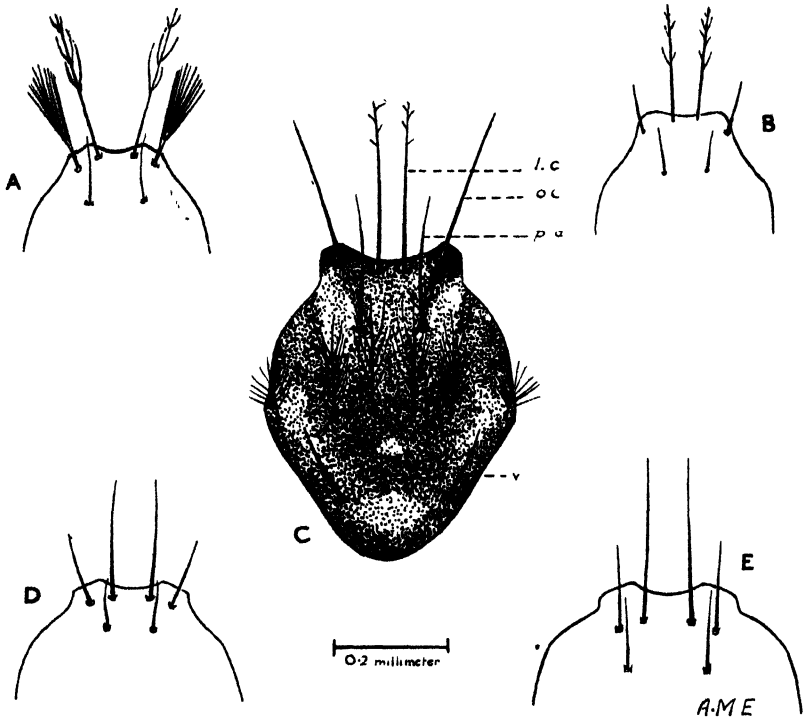


FIG. 1. Clypeal and post-antennal hairs of A.—*A. squamosus*; B.—*A. costalis*; D.—*A. rhodesiensis*; E.—*A. marshalli* var. *freetownensis*; C.—clypeus and vertex of *A. smitbii*; i.c.—inner clypeal hair; o.c.—outer clypeal; p.a.—pre-antennal; v.—vertical.

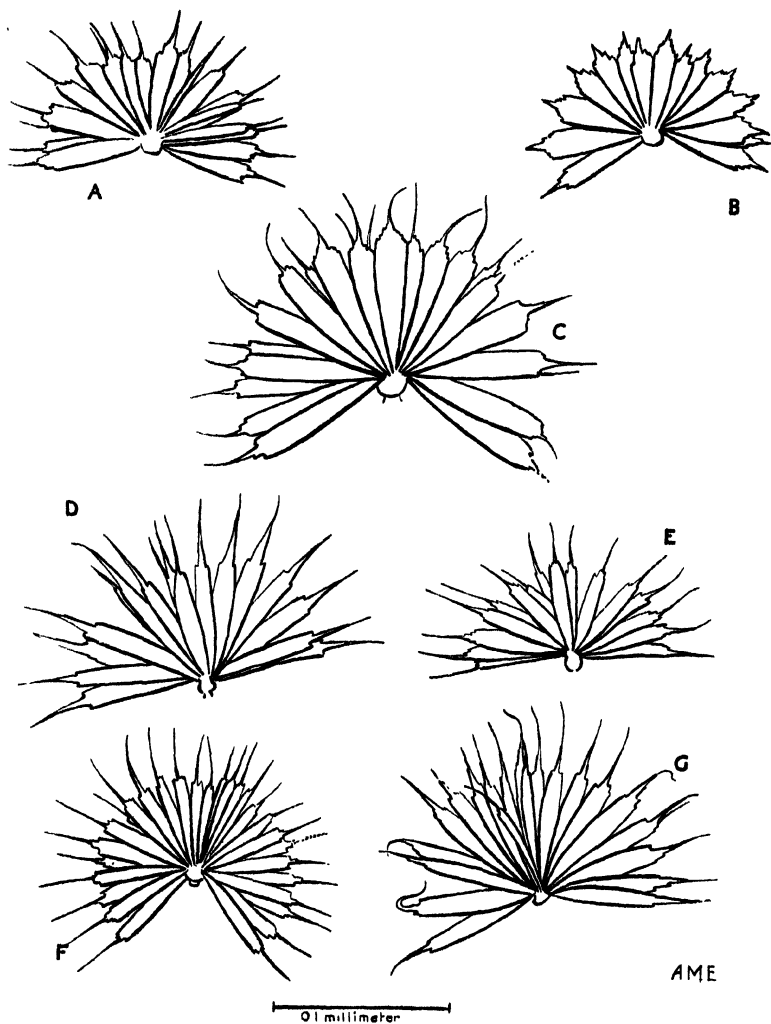


FIG. 2. Palmate hairs of fifth abdominal segment. A.—*A. rhodesiensis*; B.—*A. ibesleri*; C.—*A. marshalli* var. *freetownensis*; D.—*A. squamosus*; E.—*A. costalis*; F.—*A. funestus*; G.—*A. smithii*.

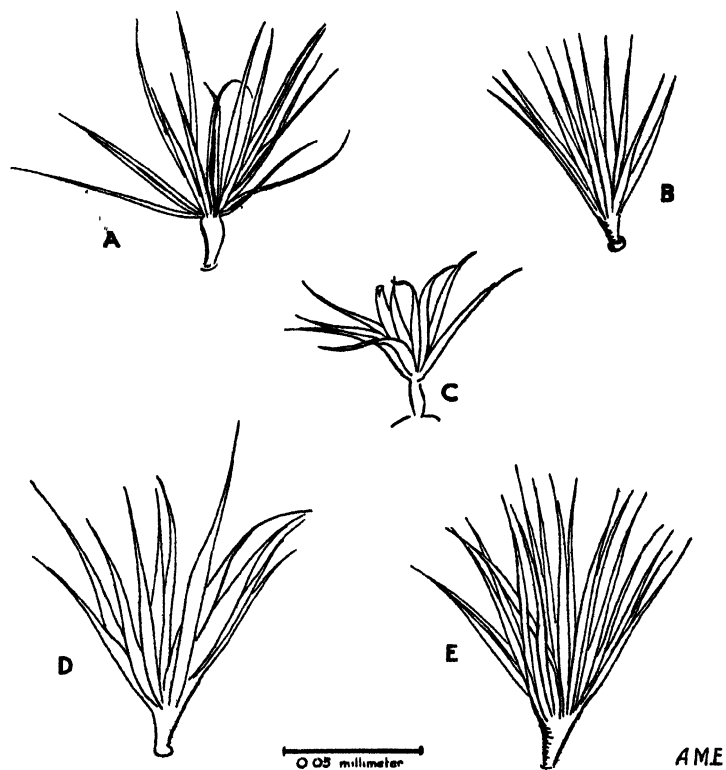


FIG. 3. Palmate hairs of thorax. A.—*A. funestus*; B.—*A. rhodesiensis*; C.—*A. squamosus*; D.—*A. smithii*; E.—*A. marshalli* var. *freetownensis*.

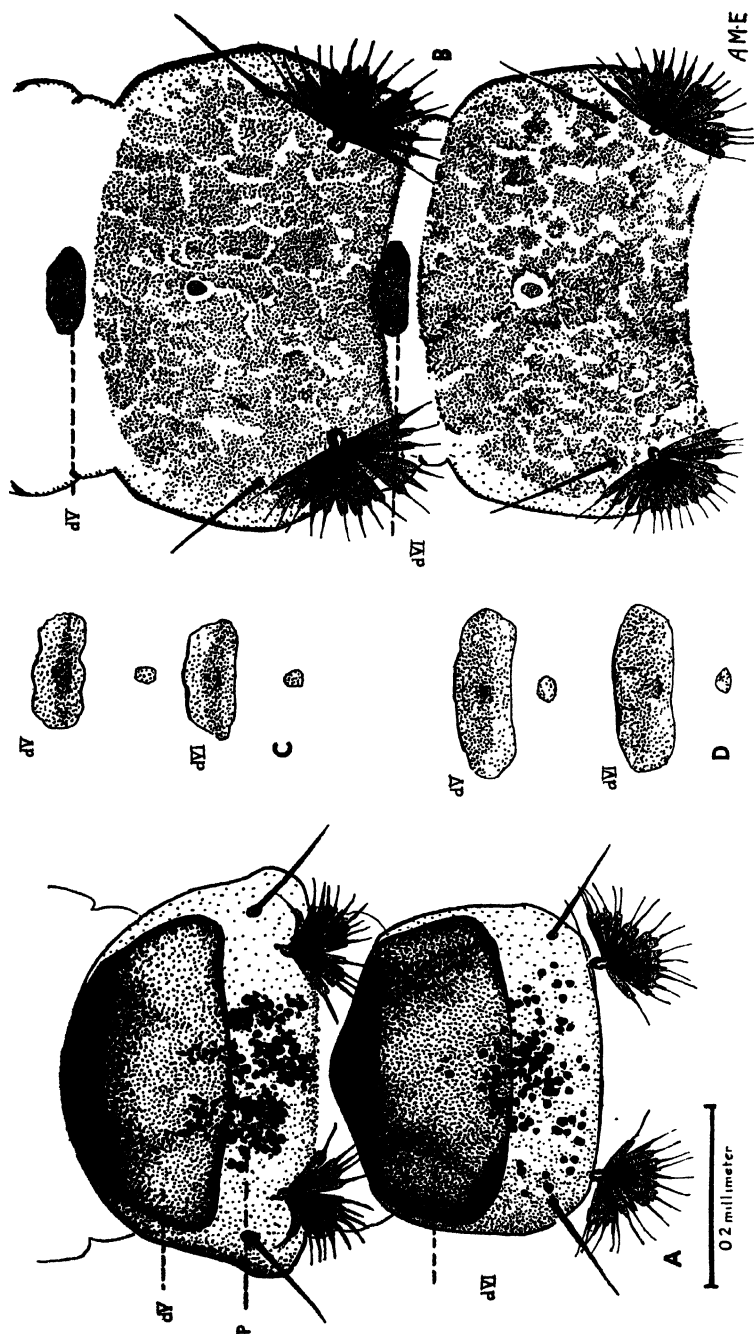


FIG. 4. A.—*A. junetui*, two abdominal segments, dorsal aspect; B.—*A. smithi*, C.—*A. marballi* var. *freitagensis*, dorsal plates of two abdominal segments; D.—*A. rhodensis*; dV, dVI—dorsal plates of fifth and sixth segments respectively; p.—pigment.

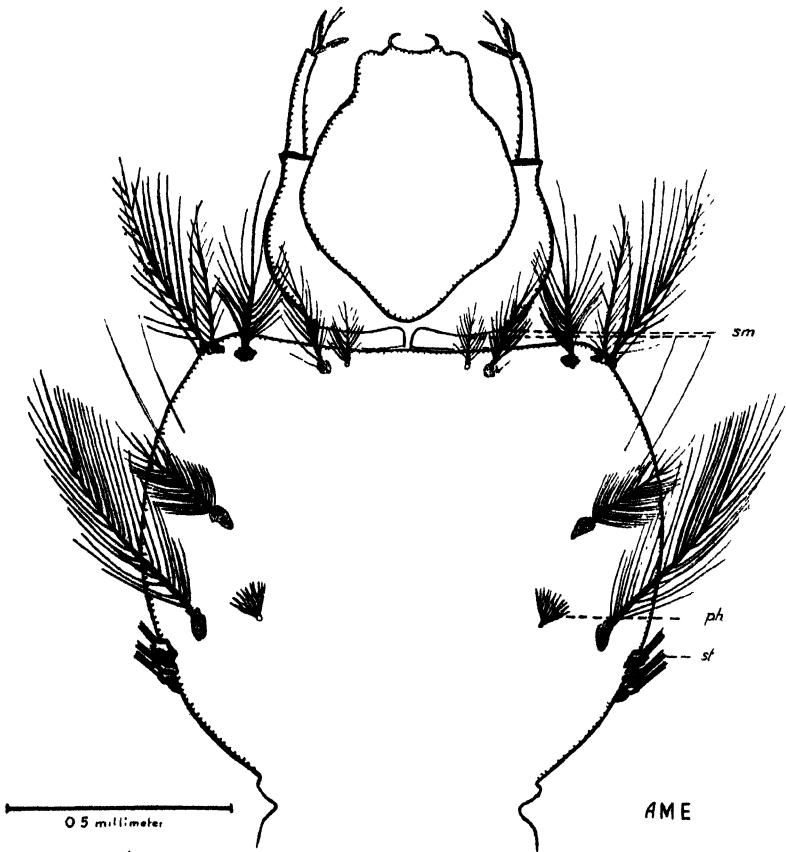


FIG. 5. *A. smitbii* (?), diagram showing position of sub-median thoracic hairs; *p.b.*—palmate hair; *s.m.*—sub-median hair; *st.*—stems of plumose hairs.

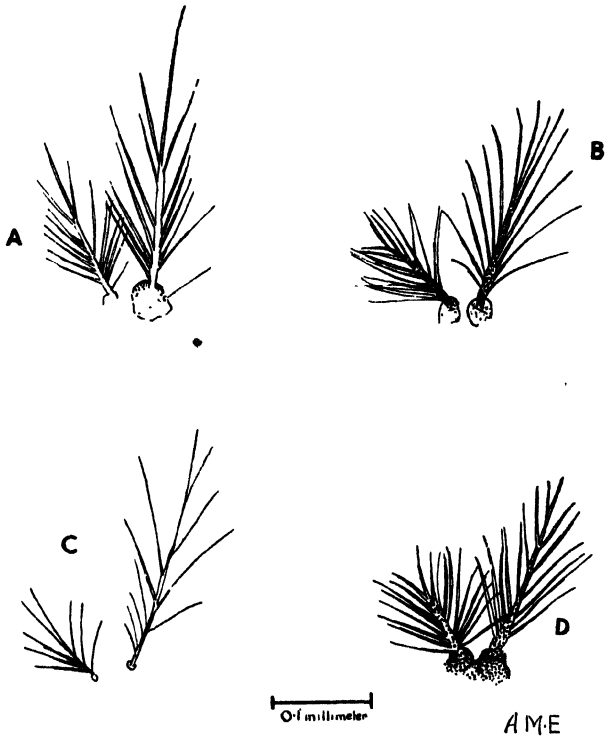


FIG. 6. Sub-median thoracic hairs of A.—*A. smitbii*; B.—*A. rhodesiensis*; C.—*A. costalis*; D.—*A. marshalli* var. *freetownensis*.



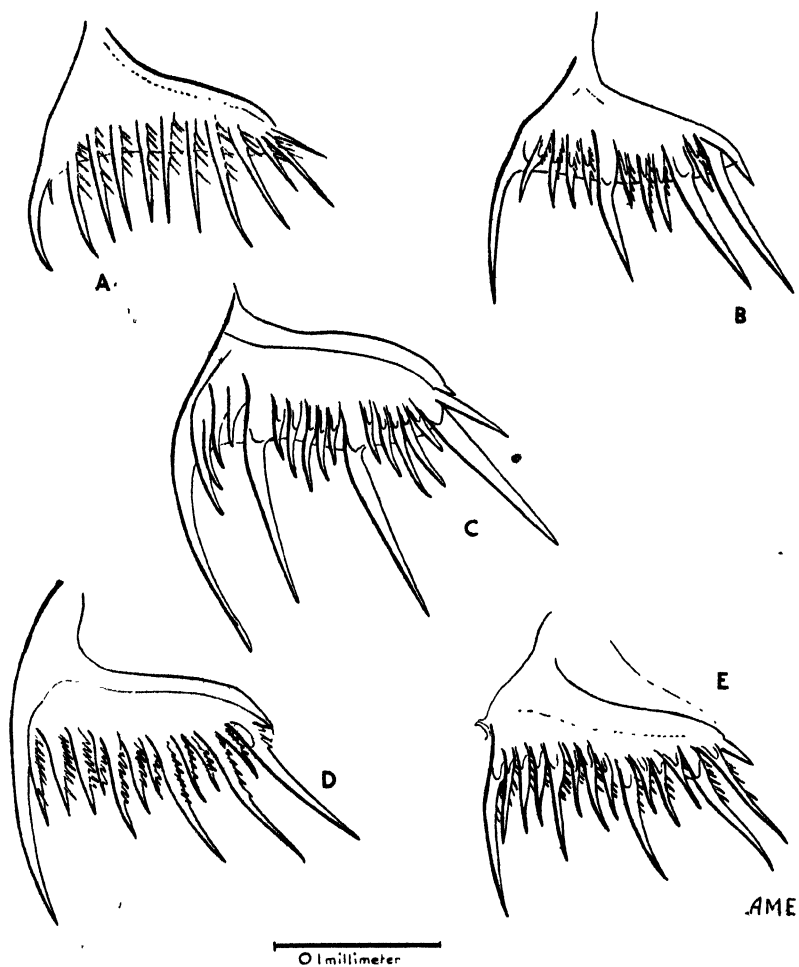


FIG. 7. Lateral combs. A.—*A. smitbii*; B.—*A. tbeileri*; C.—*A. squamosus*; D.—*A. marshalli* var. *freetoonensis*; E.—*A. rhodesiensis*.

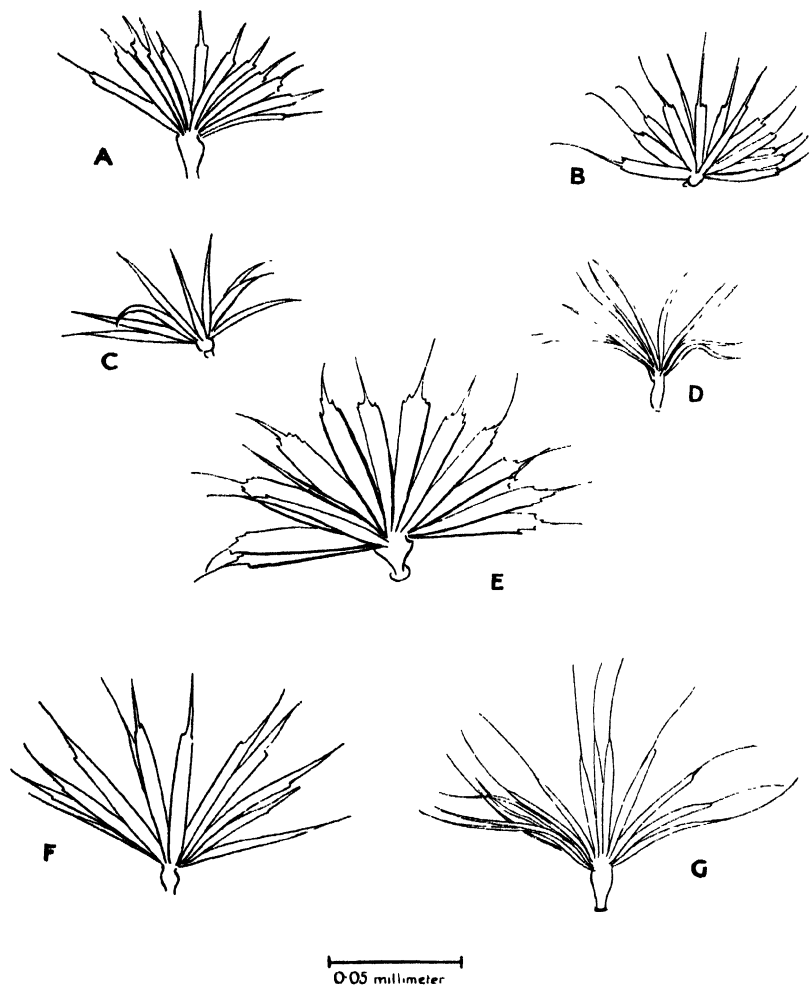


FIG. 8. Palmate hairs of first abdominal segment. A.—*A. theileri*; B.—*A. smithi*; C.—*A. rhodesiensis*; D.—*A. costalis*; E.—*A. marshalli* var. *freetownensis*; F.—*A. squamosus*; G.—*A. funestus*.

**DESCRIPTIONS OF THE LARVAE OF THREE SPECIES OF  
ANOPHELES AND NOTES ON THE DISTINCTIVE CHARACTERS  
OF OTHERS**

*A. smithii* Theo.

*Larva.* The fourth stage larva is almost invariably dark, appearing black to the naked eye. When examined microscopically the pigment is usually seen to have a bluish green hue. The clypeal hairs are very coarse, the outer are divergent and almost equal in length to the inner. The thorax bears small rudimentary palmate hairs and the lateral combs have the teeth all long and approximately equal in length, except that at the ventral end one or two short teeth occur.

*Head.* Head capsule unusually heavily chitinated, deep red-brown to black. Antennae with no branched hair on shaft, spines sub-equal, hair divided into two. Inner clypeal hairs (fig. 1, c) long, with delicate branches apically, sometimes extending backwards over the distal third. Outer clypeal hairs as stout as, and only slightly shorter than, inner clypeal, simple, divergent. Pre-antennal more than half as long as inner clypeal, stout, finely plumose; post-antennal branched; occipital simple or bifurcated. Mental plate relatively very narrow. *Thorax.* Plume hairs well developed, sub-median hairs (figs. 5 and 6, A) rather long and slender, the plates at their bases well separated. Palmate hairs (fig. 3, D) with about eleven long slender leaflets. *Abdomen.* Palmate hairs of first abdominal segment (fig. 8, B) small, but leaflets well developed with marked shoulder and filament. Palmate hairs of segments three to seven relatively large, that of fifth segment (fig. 2, G) with longest leaflets about 0.09 mm. and the greatest width about one-ninth of this. Shoulder with one, two or (rarely) three serrations, filament originating rather gradually, length (taken from distal serration to tip) about two-sevenths of the total length of the leaflet and filament. Lateral combs with about eleven sub-equal teeth, markedly serrated. Dorsal plates very small (fig. 4, B). One or two short teeth occur towards the ventral aspect.

*A. marshalli* var. *freetownensis* Evans.

*Larva, Fourth Stage.* In life the larva is usually dark grey with blackish pigment; the dorsal plates are small, and palmate hairs

are present on the thorax and first seven abdominal segments ; those of the third to seventh segments are relatively very large.

*Head.* Antennae with no branched hair on shaft ; terminal hair divided into two or three. Inner clypeal hairs (fig. 1, E) generally simple, occasionally with fine lateral secondary hairs towards the extremity. Outer clypeal hairs simple, slightly less than one-half the length of the inner ; pre-antennal, simple, about equal to the outer anterior clypeal ; post-antennal, branched, occipital simple. *Thorax.* Plume hairs well developed. Sub-median hairs (fig. 6, D) arising from chitinous plates, those of each pair being fused. Palmate hairs (fig. 3, E) large with about eighteen long narrow leaflets. *Abdomen.* Palmate hairs of first segment comparatively large (fig. 8, E) leaflets with well-developed shoulder and filament ; palmate hairs of segments three to seven unusually large, that of the fifth segment (fig. 3, E) with leaflets large (total length *c.* 0.1 mm., width from one-eighth to one-ninth of this), the shoulder much serrated and the filament long and slender, about two-sevenths of the total length of the leaflet. Dorsal plates of normal size (fig. 4, C). Lateral combs (fig. 7, D) with a rather great disparity between the long and short teeth, all the teeth finely serrated.

The larva of this species differs markedly from that of *A. marshalli* Theo., in which the first and second abdominal segments as well as the thorax are devoid of rudimentary palmate hairs (Macfie and Ingram, 1917).

#### *A. costalis* Theo.

The larva of this species has been fully described by Hill and Haydon (1907). It can usually be distinguished from the other species by the colour, the pigmented portions being pale brown or, sometimes, green, and the lateral part of the thorax opaque creamy-white. The small size of the abdominal palmate hairs (fig. 2, E) is a constant character.

#### *A. funestus* Giles.

As Edwards (1922), and subsequent authors, have pointed out, this species and its allies differ from other *Anopheles* in the larval stage by the relatively enormous size of the dorsal plates (fig. 4, A).

*A. rhodesiensis* Theo.

The larva of this species has been described by Christophers and Chand (1916).

Specimens found in the neighbourhood of Freetown are usually very dark, appearing dark grey or blackish to the naked eye, and have paired darker quadrangular areas on the abdominal segments. They may be distinguished from the larvae of *A. costalis* by the presence of small palmate hairs on the thorax (in the living larva these can be seen best by using reflected light), and by the unbranched inner clypeal hairs. The larvae are more difficult to distinguish from those of *A. marshalli* var. *freetownensis*, but the well-separated bases of the sub-median thoracic hairs can readily be seen and the large size of the abdominal palmate hairs in var. *freetownensis* is a striking character.

*A. squamosus* Theo.

Hill and Haydon (1907) have described the larva of this species in detail. The dendriform, outer clypeal hairs (fig. 1, A) distinguish it at once from the other species which have no branched hair on the shaft of the antenna. *A. pharoensis*, however, shares this character, and Edwards (1912), in his key to the larvae of the African Anophelines, does not distinguish between the two species.

*A. theileri* Edw.

There seems to be no difficulty in separating the larvae of this species from those of others likely to be met with in Sierra Leone. In addition to possessing the characters given in the key, the larva is unusual in having the smaller teeth of the lateral combs (fig. 7, B) extremely short.

*A. obscurus* Grünb.

Christophers (1924) has recently separated the African form of *A. umbrosus* Theo. under the name *A. obscurus* Grünb. From the close resemblance between the adults of the two species it seems safe to assume that the larvae of *obscurus* will have the same general characters as those of *umbrosus*.

*Anopheles nili* Theo.

The accompanying illustrations (figs. 9 and 10) show the characteristic features of a larva found in the Moa River, at Daru, by the senior author in 1924.

The larva was one of several taken from the edge of the river, many of which hatched out, giving rise to two species only, namely, *A. costalis* and *A. nili*. There is, therefore, very little doubt that this larva is that of *A. nili*. A similar but slightly imperfect larva was found in a swamp near Daru. The following is a description of the larva.

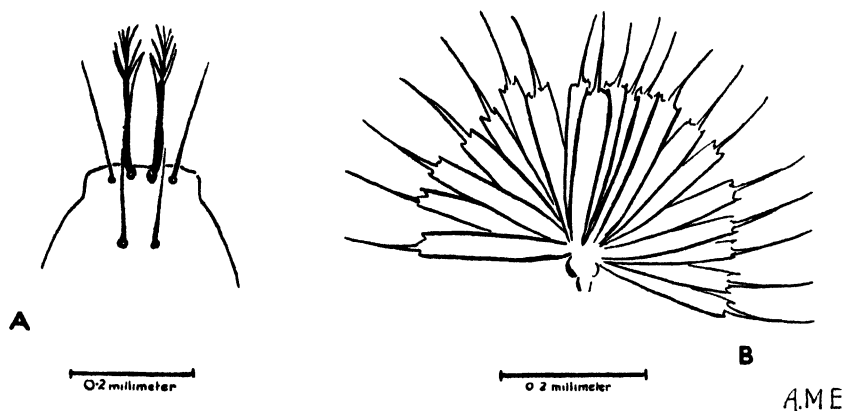


FIG. 9. *Anopheles* sp ? *nili*. A.—clypeal and post-antennal hairs; B.—palmate hair of thorax.

*Head.* Antennae with no branched hair on shaft. Inner clypeal hairs (fig. 9, A) very thick, widest a short distance beyond the base, distal third with several rather long coarse branches. Outer clypeal hairs slender, simple. Pre-antennal hairs reaching to the edge of the clypeus, simple. *Thorax.* Palmate hairs (fig 9, B) situated at the posterior angles, very large, *c.* 0.14 mm. in diameter, the leaflets with well-developed shoulder and filament. *Abdomen.* Palmate hairs (fig. 10, B) relatively large, the leaflets with filament and rounded, serrated shoulder, markedly incised between serrations. (It should be noted that the palmate hairs in figs. 9 and 10 are drawn to different scales.) Dorsal plates (fig. 10, A) wide, resembling in shape the thicker basal portions of the dorsal plates of *A. funestus*. Lateral combs with long and short teeth.

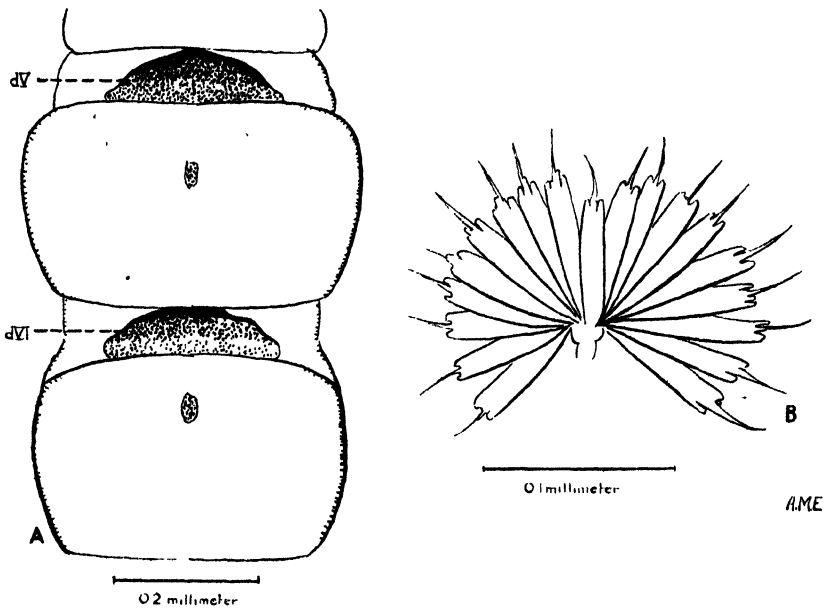


FIG. 10. *Anopheles* sp. ? *nil*i. A.—outline of two segments of abdomen; B.—palmate hair of fifth abdominal segment; *dV*, *dVI*.—dorsal plates of fifth and sixth segments respectively.

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## EXPLANATION OF PLATE V

## Breeding places of Anopheles.

- FIG. 1. Breeding place of *A. rhodesiensis* in the town. The white arrow is pointing to a small pool from which larvae were taken.
- FIG. 2. Breeding place of *A. smithii* above Aureole Bridge. The small rock-pool indicated by the arrow contained larvae of this species.
- FIG. 3. Breeding place of *A. funestus* just outside the town. The lower barb of the arrow is pointing to the floating weeds among which the larvae were found.
- FIG. 4. Breeding place of *A. rhodesiensis* and *A. costalis* in the town.



1.



2.





# THE CULTIVATION OF *ENDAMOEBA* *RANARUM*\*

BY

HARVEY P. BARRET

AND

NANNIE M. SMITH

(Received for publication 10 November, 1925)

When the present authors began the work on the cultivation of the intestinal amoebae in the cold-blooded vertebrates in 1922, it was hoped that the work would not only be of interest in itself, but might throw some light on the cultivation of the forms from man. At that time the only apparent successful cultivation of a species of *Endamoeba* was the work of Cutler, 1918, on *E. histolytica*, and his work was not accepted by many protozoologists. We succeeded first in cultivating an *Endamoeba* from the turtle (Barret and Smith, 1924). In a footnote to the same paper we noted that we had carried one strain of *E. ranarum* for more than two months. We considered the cultivation of *E. ranarum* to be of especial interest because of the great similarity in structure between this form and *E. histolytica* of man. Since our work began, Boeck and Drbohlav (1925) have undoubtedly succeeded in cultivating the parasite of amoebic dysentery, and Chiang (1925) has cultivated a very similar form (*E. histolytica* var. *murina*) from the rat. Drbohlav (1925, a, b, and c) also reports the cultivation of *E. gingivalis*, *E. coli* and *E. aulastomi*. The object of the present paper is to give the details of the successful cultivation of *E. ranarum* from tadpoles.

\* From the Laboratory of Dr. H. P. Barret, Charlotte, N.C., and the Department of Hygiene and Bacteriology, University of Chicago.

### CULTURE MEDIUM USED

The culture medium used is the same as that described in an earlier paper in the cultivation of *Blastocystis* (Barret, 1921). It consists of one part of inactivated human serum and nine parts of 0.5 per cent. salt solution.

The H-ion concentration of the medium varies from 7.6 to 7.8 in its natural state. Experimentally it was found that the amoebae would grow in media, ranging from pH 5 to pH 10, but that the most favourable range is between 7 and 8.

### PROCEDURE

The lower end of the large intestine of a tadpole is removed, placed on a sterile slide and covered with a small quantity of the culture medium. The contents of the intestine are expressed, and after being mixed with the culture medium, are drawn up in a capillary pipette and placed in two or more tubes containing the medium to a depth of about 50 mm. The material is always placed in the bottom of the tubes as growth takes place only in that portion. The tubes are then placed in the icebox and are allowed to remain undisturbed for from ten days to two weeks, and in some instances longer, after which the sediment is examined for amoebae. One of the mistakes made in the initial attempts to culture the amoebae was an examination of the sediment after too short an incubation period, a fact which may account, in part at least, for the low percentage of positive cultures in our earlier work. When a positive culture is found, transplants are made into several tubes every week or two, depending on how rapidly the amoebae grow. In our routine procedure transplants were never made under one week and some strains did better if left undisturbed for two or even three weeks.

Many obstacles were encountered, which added greatly to the labour of the work. At first, the contents of the lower end of the large intestine were only sub-cultured if they were microscopically found positive for amoebae. Experience showed that this procedure was detrimental to the amoebae, because of drying, etc. Instead, the contents of each intestine were treated exactly as if they contained amoebae and microscopical examination was deferred until the appropriate time for sub-culturing. In the second place, infected frogs and tadpoles are extremely scarce. Some observers have

found as high as 5 per cent. of certain species of frogs infected, while others place the figure at 1 per cent. Owing to this, as well as to the fact that tadpoles are handled in large quantities more easily than frogs, the present workers used tadpoles exclusively. Out of five hundred tadpoles examined, we obtained only six positive cultures, or a little over 1 per cent. infection. In the third place, there were many other organisms in nearly every animal examined which, because of their much more rapid growth, inhibited the multiplication of the amoebae. *Blastocystis* and intestinal flagellates of different kinds were the most troublesome contaminants. In fact, if a culture was contaminated with *Blastocystis* it could neither be freed from them nor successfully transplanted many times thereafter. On the other hand, one of us (Smith) has met with a certain amount of success in freeing cultures of flagellates by subjecting them to varying dilutions of mercurochrome for different lengths of time. The most favourable dilution of mercurochrome was 1-2000; the most favourable time of exposure was one hour.

The writers have been able to carry three strains of *E. ranarum*, obtained from tadpoles 220, 222, and 226 respectively, continuously, through numerous sub-cultures, for more than eight months. Furthermore, these strains at their last transfer showed no evidences of dying, and hence, can apparently be kept going indefinitely.

Cysts were present in practically all cultures after a week's growth, their numbers probably depending on the age of the culture. There seems to be, however, no tendency for the active amoebae to disappear entirely in the very old cultures and their places to be taken by cysts. When a culture died, the cysts and amoebae soon disappeared. In this respect, cultures of *E. ranarum* differ from cultures of free-living amoebae, for in the latter, cultures are viable for months owing to the presence of the cysts alone. As stated above, we have been able to obtain cultures that are free of other protozoa; but all cultures are contaminated with the associated bacteria. From findings in some of the cultures, we believe there is more than one species of amoebae living in the frog. We hope to take up this question later.

A description of the morphology of the amoebae in our cultures is given in the paper by Taliaferro and Fisher, immediately following this one.

### CONCLUSION

*E. ranarum* has been successfully cultivated on a simple medium, and strains have been carried through successive transplants over a period of more than eight months.

### LITERATURE CITED

The literature cited in the present paper is given at the end of the paper by Taliaferro and Fisher, which follows.

# THE MORPHOLOGY OF MOTILE AND ENCYSTED *ENDAMOEBA RANARUM* IN CULTURE

BY

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AND

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(*Received for publication 10 November, 1925*)

## PLATE VI

All of the amoebae used in this study were obtained from cultures started and furnished us by Barret and Smith. These investigators originally started their cultures from amoebae found in tadpoles and a detailed description of their technique will be found in their paper, immediately preceding this one. The present paper indicates beyond reasonable doubt that these authors have successfully cultivated an *Endamoeba* which, in culture, goes through the usual stages observed in the body, viz., active amoebae, precystic amoebae and cysts, and furthermore, that the specimens in all stages of development are identical with the description given by various authors of *Endamoeba ranarum* Grassi, 1879. In our previous study (Taliaferro and Holmes, 1924) of the morphology of an entozoic amoeba from the turtle, which Barret and Smith had cultivated, we deemed it necessary to study the forms from both the turtle and cultures. In the present case, however, where the form with which we are dealing has been studied by many investigators who have given excellent descriptions of it, it seemed necessary to consider only the amoebae from the cultures.

At the outset we wish to emphasize that a morphological study is always essential before an author is justified in concluding that he has actually cultivated an entozoic species. In fact, omissions of



this kind have been the basis of much confusion in the earlier attempts to cultivate entozoic amoebae. Thus, free-living contaminants have been mistaken for the entozoic species which were supposed to have been cultivated.

Since a review of the earlier work may be found in Dobell (1909) and much of the later work in Nöller (1922), no attempt will be made to review the various papers on the morphology of *E. ranarum*. Suffice it to say that Dobell has given an accurate description of the organism—a description which agrees in all major details with that given in the present paper. Moreover, not only can we compare the present cultural forms with the description by Dobell, but we can also make use of the resemblance between *E. ranarum* and *E. histolytica*. This will be most helpful, since the structure of *E. histolytica* has been so widely studied and is so well known. Dobell, in a footnote to the paper cited, calls attention to the extraordinary 'resemblance' between Hartmann's figures of *E. tetragena* (= *E. histolytica*) and isolated stages in *E. ranarum*. This remarkable resemblance between *E. histolytica* and the parasite of the frog has been noted by several subsequent workers. It even led Alexeieff (1914) to suggest that the harmless commensal of the frog, when introduced accidentally into man, might become the parasite of amoebic dysentery. The infection experiments of Dobell (1918) invalidate this conclusion, however, and indicate that the two species are distinct. With regard to the morphological similarity of the two, Dobell (1918) states, 'The active amoebae can usually be readily distinguished from one another by the inclusions (food bodies) in their protoplasm, but not by their own nuclear and cytoplasmic structure; but the precystic amoebae, devoid of all food bodies, and the cysts, at every stage of development, are so closely alike that preparations of the one could be used as demonstrations of the other.'

## MATERIAL AND METHODS

As previously stated, all of our material was supplied us in culture by Barret and Smith. These cultures were subcultured on Barret and Smith's medium in this laboratory for several months. In the previous work of Taliaferro and Holmes (1924) on *Endamoeba barretti*

from the turtle, it was found that rabbit serum or Loeffler's dehydrated beef serum could be substituted for human serum in the original Barret medium. In our rather limited experience with *E. ranarum*, on the other hand, we have not obtained anything like as satisfactory results with rabbit or pig serum, Loeffler's dehydrated beef serum or human ascitic fluid, as we did with human serum.

All observations of living specimens were carried out at room temperature with material mounted under cover glasses sealed with vaseline. All of the prepared slides were fixed in Schaudinn's alcohol-sublimated mixture with 2 per cent. acetic acid. They were stained with Delafield's or Heidenhain's iron-haematoxylin with and without counter stains. No fixative was used to get the amoebae to adhere to the slide. As a consequence, the larger cysts tended to wash off the slides during the process of staining and dehydration (see differences in measurements of living and prepared cysts).

We have made a very careful study of the size of the present form in all three stages of its development, viz., active and precystic amoebae and cysts. In all cases the forms were drawn with a camera lucida, generally at a magnification of  $\times 3,000$ , and the drawings measured. Owing to the characteristic circular shape of the precystic amoebae and the cysts, their size is given as their diameter in microns, but, since the active amoebae are generally very irregular in outline, their measure of size is given as the diameter of a circle having approximately the same area as the amoeba. (This method is similar to that used by Taliaferro and Holmes on *E. barreti*.)

With regard to size, active amoebae were measured from prepared slides; (1) from a culture containing only active forms, i.e., containing no cysts; (2) from a culture in which cysts had just begun to appear, and (3) from a month-old culture in which there was a large number of cysts. Their range in size varied as follows:—

No. measured	Range	Average
(1) 60	18·3 $\mu$ to 38·0 $\mu$	26·3 $\mu$
(2) 53	12·0 $\mu$ to 38·5 $\mu$	23·1 $\mu$
(3) 45	13·2 $\mu$ to 26·0 $\mu$	19·0 $\mu$

The progressive decrease in average size which is brought out by these measurements is just what would be expected, since parasitic amoebae habitually grow smaller in preparation for encystment. In

passing, we may note that the size of these forms is within the range of size recorded for *E. ranarum* in the literature. Furthermore, the size of the large active amoebae ( $18.3\mu$  to  $38.0\mu$ , average  $26.3\mu$ ) is quite similar to the size of *E. histolytica*. Dobell (1919) gives the usual range of *E. histolytica* as  $20\mu$  to  $30\mu$ , and the extreme range as  $18\mu$  to  $40\mu$ .

The range in size of cysts studied in iodine was  $9.6\mu$  to  $20.6\mu$ , with an average of  $14.8\mu$ . Later, 105 cysts from the same culture, but drawn from prepared slides, showed a range of  $6.3\mu$  to  $14.5\mu$ , with an average of  $10\mu$ . In view of Dobell and Jepp's (1918) study of the size of *E. histolytica*, we might have expected a slight decrease in diameter (about 10 per cent.), but nothing like the one observed. We believe that the discrepancy is due simply to the fact that the smaller cysts adhere to the slide better than the larger ones. Indeed, this is borne out by the fact that in destaining the slides, one can actually see the large cysts being washed off. Therefore, we are probably justified in giving the range of size of the cysts in our cultures as from about  $6\mu$  to  $20\mu$ . The great similarity of these measurements to those of both *E. ranarum* and *E. histolytica*, is apparent when it is recalled that the diameter of cysts of *E. ranarum* is given as  $10\mu$  to  $16\mu$  by Nöller (1922) and of *E. histolytica* as  $5\mu$  to  $20\mu$ , by Dobell (1919).

The appearance of the active amoebae in the cultures depends largely on the food available. In the first cultures which contained flagellates, the endoplasm was generally so loaded with these organisms as to obscure the nucleus. In later cultures which were free of flagellates the motile amoeba appeared much like that shown in fig. 1. Specimens, for some time after being placed on a slide, assumed an elongated shape and progressed by means of lobose pseudopodia. These were extruded as masses of clear ectoplasm into which, later, the endoplasm flowed, and were formed from alternate sides of the anterior portion of the animal (fig. 1). When the slide began to dry up, the amoebae generally assumed a more spherical shape and extruded—almost explosively—clear hyaline pseudopodia without any evidence of active progression. In this condition they were almost identical in appearance with *E. histolytica* as seen in ordinary mounts of fresh faeces. The nucleus could be generally seen as a ring of dense material in which was embedded a

number of bright refractile granules (fig. 1). The karyosome was rarely visible in the living specimen although it was visible in the specimen from which fig. 1 was drawn.

The general appearance of the active amoebae in stained preparations is shown in fig. 6, and the nuclei of two other specimens in figs. 7 and 8. The endoplasm of the specimen in fig. 6 was crowded with food vacuoles containing flagellates. The structure of the nucleus is interesting because it possesses all of the distinguishing characteristics of *E. histolytica*, such as the delicate layer of chromatin around the periphery, the small centrally-placed karyosome which is shown in figs. 7 and 8, and the linin network between the karyosome and periphery which is devoid of chromatin. In deeply-stained specimens, the karyosome cannot be seen (fig. 6)—most probably owing to its being obscured by the overstained linin network.

The large active amoebae frequently have more than one nucleus. In the sixty amoebae drawn to give the measurements discussed in a previous paragraph, six possessed two nuclei and one possessed four.

At no stage do any of the specimens ever show any trace of a contractile vacuole.

In cultures at the height of their growth there is little or no tendency for the amoebae to encyst, but as the cultures grow older the amoebae become smaller and more sluggish. In time these forms lose all food inclusions and become typical precystic amoebae. Fig. 9 shows a specimen which is probably intermediate between the large active form and the precystic form, whereas fig. 10 shows a typical precystic amoeba. These are even more like *E. histolytica* than the active forms. Their nuclei, as shown in fig. 10, are in every respect identical with that of *E. histolytica*, and in our experience, both the karyosome and achromatic capsule are more clearly seen than in the motile forms.

When the precystic forms encyst they are again identical with the cysts of *E. histolytica*. Figs. 2, 3 and 4 show their general appearance when alive. Quite frequently one or two nuclei can be made out in the living cysts, but it is rare to see all four, and sometimes none are visible. One nucleus is barely discernible in figs. 2 and 3, respectively. The chromatoid bodies appear rodlike and the glycogen masses as dull inclusions. The nuclei can be easily counted when the cysts are observed in iodine or stained with iron-

haematoxylin. Fig. 11 shows a cyst with one nucleus which is probably in an early stage of division, and figs. 12, 13 and 14 show mature quadrinucleate cysts. Each of these contain chromatoid bodies so diagrammatically like *E. histolytica* as to need no further description. Of the several hundred cysts examined from culture, none contained more than four nuclei, a condition once more similar to *E. histolytica*, for although there is some evidence that *E. histolytica* occasionally forms a supernucleate cyst with eight nuclei, the occurrence must be extremely uncommon. We have never encountered in our cultures any cysts suggesting the figures of Mercier and Mathis (1918), in which they depict their so-called "schizogonic" cysts.

### SUMMARY

1. A detailed description is given of the amoebae from cultures originally isolated, by Barret and Smith, from tadpoles. The structure of these amoebae agrees in minutest detail with the descriptions by other authors of *Endamoeba ranarum* from the frog.
2. In culture, *E. ranarum* goes through the typical development of a parasitic amoeba, eventually forming cysts.
3. In agreement with all recent investigators, the present investigation emphasizes the similarity in structure of *E. ranarum* and *E. histolytica*.

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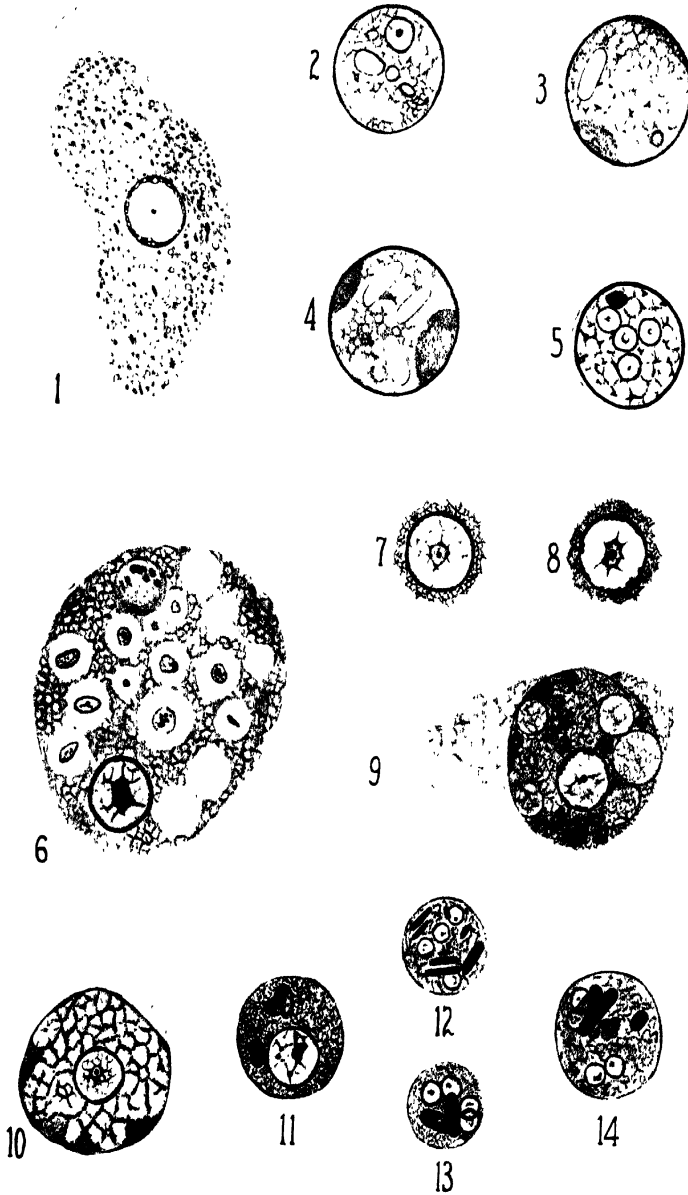
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## EXPLANATION OF PLATE VI

All figures are of *Endamoeba ranarum* from cultures in Barret's medium, reproduced at a magnification of  $\times 1400$ . All drawings were made from camera lucida sketches, although that of the living motile amoeba (fig. 1) was necessarily largely a free-hand drawing. Stained specimens (figs. 6-14) were all fixed in Schaudinn's fluid and stained with Delafield's iron-haematoxylin without counter stain.

- Fig. 1. Active amoeboid form as seen in living condition from a culture which had been freed of all other species of protozoa. The endoplasm contains a number of rod-like bacteria. Note that the karyosome of the nucleus is visible, although this is an exceptional occurrence in the living organisms.
- Figs. 2, 3 and 4. Cysts from culture as seen in the living condition. Note that each cyst contains chromatoid bodies; that figs. 3 and 4 contain one and two glycogen masses, respectively; and that in figs. 2 and 3, a nucleus is visible.
- Fig. 5. A cyst in iodine from the same culture as the one shown in fig. 2. Note the four nuclei. No chromatoids are visible but there is a rather lightly-stained glycogen mass indicated near the top of the cyst.
- Fig. 6. Active amoeboid form fixed in Schaudinn's fluid and stained with Delafield's iron-haematoxylin. This form came from a culture which contained intestinal flagellates. The food vacuoles contain débris from the digestion of these flagellates. A definite karyosome is not seen in this specimen.
- Figs. 7 and 8. Nuclei of active amoeboid forms fixed and stained in the same manner as the specimen shown in fig. 6. A karyosome is visible in each.
- Fig. 9. An amoeboid form (fixed and stained as noted) which is probably intermediate between the large active amoebae and the precystic forms.
- Fig. 10. A typical precystic amoeba. (Fixed and stained as noted.)
- Fig. 11. A uninucleate cyst. Four chromatoid bodies are present and the nucleus is probably in a very early anaphase. (Fixed and stained as noted.)
- Figs. 13 and 14. Mature quadrinucleate cysts, all of which contain chromatoid bodies. (Fixed and stained as noted.)







# NOTES ON FREETOWN MOSQUITOS, WITH DESCRIPTIONS OF NEW AND LITTLE-KNOWN SPECIES

BY

A. M. EVANS

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## PLATE VII

The material dealt with in this paper was collected in the course of a recent survey of the mosquitos of Freetown by Professor D. B. Blacklock and the writer. During the survey very valuable assistance was rendered by Dr. H. O'Hara May, Deputy Director of Sanitary Services, and by the officials of the Sanitary Department, who submitted to us for identification about three hundred consignments of larvae, many of them from tree-holes in Freetown and at Hill Station. I am also indebted to Dr. M. G. Blacklock, Dr. R. M. Gordon and Dr. G. MacDonald, for specimens of larval and adult mosquitos.

The following non-anopheline mosquitos are recorded :—

- |  |  |
|--|--|
| <i>Culex albiventris</i> Edw.                  | <i>A. (Aedimorphus) simulans</i> Newst., and       |
| <i>C. annulioris</i> Theo.                     | Carter   |
| <i>C. decens</i> Theo.                         | <i>A. (Aedimorphus) tarsalis</i> Newst.            |
| <i>C. decens</i> var. <i>invidiosus</i> Theo.  |  |
| <i>C. duttoni</i> Theo.                        | <i>Uranotaenia balfouri</i> Theo.                  |
| <i>C. grahami</i> Theo.                        | <i>U. conalli</i> Edw.                             |
| <i>C. horridus</i> Edw.                        | <i>U. fusca</i> Theo.                              |
| <i>C. (Culicomyia) nebulosa</i> Th.            | <i>U. nigripes</i> Theo.                           |
| <i>C. (Culicomyia) cinereus</i> Theo.          | <i>U. ornata</i> Theo.                             |
| <i>Lutzia tigripes</i> var. <i>fusca</i> Theo. | <i>Hodgesia sanguinis</i> Theo.                    |
| <i>Aedes (Stegomyia) africana</i> Theo.        | <i>Ficalbia mediolineata</i> Theo.                 |
| <i>A. (Stegomyia) argenteus</i> Poirct.        | <i>Harpagomyia trichorostri</i> Theo.              |
| <i>A. (Stegomyia) blacklocki</i> Evans         | <i>Megarhinus brevipalpis</i> Theo.                |
| <i>A. (Stegomyia) frasci</i> Edw.              | <i>M. aeneus</i> n.sp.                             |
| <i>A. (Stegomyia) luteocephala</i> Newst.      | <i>M. ? phytophagus</i> Theo.                      |
| <i>A. (Stegomyia) simpsoni</i> Theo.           | <i>Eretmopodites chrysogaster</i> Graham           |
| <i>A. (Stegomyia) vittata</i> Bigot            | <i>E. chrysogaster</i> var. <i>semisimplicipes</i> |
| <i>A. (Finlaya) longipalpis</i> Grünb.         | Edw.   |
| <i>A. (Aedimorphus) albocephalus</i> Theo.     | <i>E. dracaenae</i> Edw.                           |
| <i>A. (Aedimorphus) apicoannulata</i> Edw.     | <i>E. inornatus</i> Newst.                         |
| <i>A. (Aedimorphus) domesticus</i> Theo.       | <i>E. leucopus</i> Graham                          |
| <i>A. (Aedimorphus) occidentalis</i> n.sp.     | <i>E. oedipodius</i> Graham                        |



*Aedes (Aedimorphus) occidentalis* n.sp.

*Aedes apicoannulatus* Edwards, *Trans. Roy. Soc. Trop. Med. and Hyg.*, Vol. XVI, p. 500.

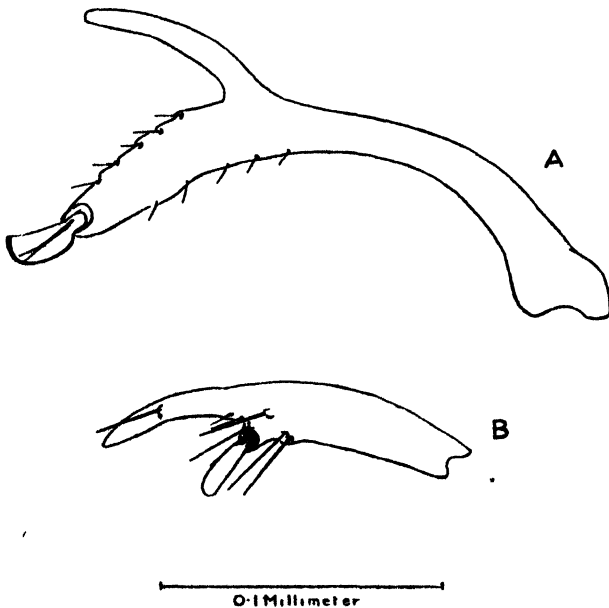
*Ochlerotatus apicoannulatus* Ingram and Macfie, *Bull. Ent. Res.*, Vol. VIII, p. 144.

It was found that amongst the large numbers of *Aedimorphus* with apically banded tarsi there were two distinct species in addition to *A. simulans*, N. and C., which has characteristic mesonotal spots of narrow, silvery scales. One of these species was entirely without white scales on the thorax; it was present in numbers enormously greater than the other species, which possessed antero-lateral stripes of flat white scales on the dorsal surface of the thorax. The side-pieces of the male hypopygium were quite distinct in the two species and the larvae exhibited striking differences. The less common species with the thoracic stripes has hitherto usually been identified with *apicoannulata* Edw., but Mr. Edwards, who kindly compared examples of both species with Theobald's type of *Aedimorphus alboannulatus* (Edwards bestowed the new name "*apicoannulata*" in 1912, *alboannulatus* being preoccupied), informed me that the commoner mosquito with unornamented thorax agreed with the type. The rarer species must, therefore, be regarded as new and the name *occidentalis* is proposed, as this species is the western representative of two closely-allied African species (Edwards, 1923, p. 500). As the characteristics of this mosquito have been referred to by Edwards (1923, 1925), a full description does not seem necessary. The new species differs from *A. apicoannulatus* Theo. chiefly as follows:—The proboscis is entirely dark scaled; there are large paired patches of broad silvery scales immediately behind the eyes; in *A. apicoannulatus* the pale scales in this position are very narrow and yellowish; the mesonotum is adorned with paired stripes of silvery scales on the anterior margins, extending backwards to the scutal angle, the stripes consisting in front of two layers of outwardly directed flat scales and, behind, of irregularly arranged flat scales directed obliquely backwards. Dark scales of mesonotum unmixed with paler ones; in *apicoannulata* there is an admixture of yellowish-brassy scales among the dark ones of the mesonotum, but no definite pattern formed by pale scales. The male hypopygium

shows striking differences from that of *apicoannulata*; the claspers have been figured by Edwards (1923), but they are illustrated here (fig. 1, B) for comparison with those of that species. The larva has been fully described by Ingram and Macfie (1917); the chief differences between it and the larva of *apicoannulata* are tabulated on p. 102. Type ♀ and two cotype ♂♂ reared from larvae found in tree-holes, Freetown, 6,VIII.25, by officials of the Sanitary Dept., Sierra Leone.

*A. (Aedimorphus) apicoannulata* Edw.

This species appears to be by far the commonest tree-hole breeding mosquito in Freetown, at any rate during the wet season. The male hypopygium differs from that of *A. occidentalis* n.sp., in the form of the claspers (fig. 1, A and B), and in the possession of rudimentary claspettes, partially fused with the internal surface of the side-pieces,



A.M.E.

FIG. 1. Clasper of male hypopygium. A—*A. apicoannulata*;  
B—*A. occidentalis*.

and bearing two strong spines at their extremities. The clasper in *A. simulans*, N. and C., is not distinguishable from that of *apicoannulata*, but the claspettes of the former species bear hairs instead of spines.

*Larva.* Fourth stage.

*Head.* Antenna curved, with a sub-median tuft of five hairs, and shaft sparsely spinose. Mid frontal hairs plumose. Mental plate (fig. 2, D) with a median tooth and fourteen teeth on each side, of which the inner seven or eight are very small and close together. First and second segments of abdomen with very stout, plumose, multiple hairs laterally, that on the first with four or five, that on the second with three or four branches. Lateral combs (fig. 2, A, C) consisting of sub-triangular patches of very numerous

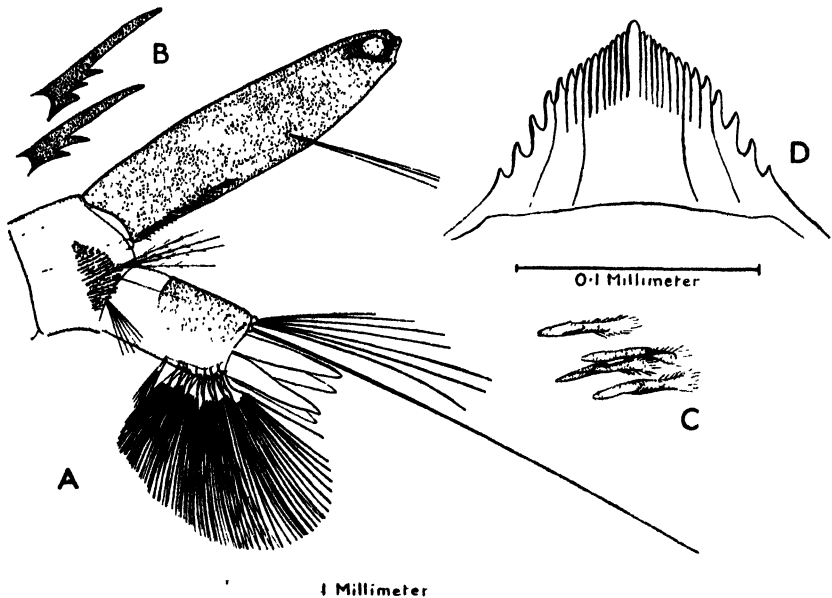


FIG. 2. *A. apicoannulata*, larva. A—eighth and ninth segments; B—pecten spines; C—comb spines; D—mental plate; B and C to the same scale as D.

(about eighty) small, elongate fringed spines. Siphon tube with the length four times the greatest width; pecten consisting of nineteen barbed teeth; tuft of two or three long hairs, reaching to or slightly beyond the apex of the siphon. Dorsal hairs of anal segment consisting of a tuft of seven hairs, and a hair arising from a separate base exceeding twice the length of the longest hairs of the tuft.

Table showing chief differences between the fourth stage larva of *A. apicoannulata* and *A. occidentalis*.

	<i>A. apicoannulata</i> Edw.	<i>A. occidentalis</i> n.sp.
Mental plate ... . . . .	With 19 teeth	With 29 teeth
Lateral combs ... . . . .	11-12 spines in an irregular row	About 80 spines in a sub-triangular patch
Tuft of siphon tube ... . .	6 hairs	2-3 hairs
Dorsal hairs of anal segment ...	3 and 1	7 and 1

*Megarhinus (Toxorhynchites) acneus* n.sp.

**FEMALE. Head.** Occiput with brilliant peacock-blue scales in front, and narrow, pale greenish scales with golden reflections behind and at the sides; a border round the eyes narrow and purplish above, broader and white below. Proboscis and palpi with deep violet scales, those of palpi with bright blue reflections at the apices of the segments. **Thorax.** Prothoracic lobes with golden bristles and metallic blue scales with violet reflections above, and darker bristles and ochraceous golden scales below. Mesonotum with bright yellow setae in front, scales dull green appearing bronzy in certain aspects, brighter and more bluish-green scales on and near the scutellum and a small patch of bright blue scales over the wing root. Setae of scutellum and above wing root deep golden; pleurae golden yellow with flat, white scales forming a broad median longitudinal band; scales above and below this band yellow with pale green iridescence. **Abdomen.** Dorsum with first segment clothed with metallic green scales with yellow reflections, rest of segments with metallic violet-pink scales with coppery reflections, lateral edges of tergites with small basal areas of paler metallic scales; sixth and seventh segments with small apical, lateral tufts of flame-coloured setae. Venter brilliant golden with pink and green reflections, seventh segment with broad median distal area of dark purplish-brown scales. **Legs.** All femora golden internally almost to the apex, and on the basal third or half externally, a line of golden scales extending on to the dark area for most of its length. Front tibia with dark metallic scales, middle tibia pale scaled on basal three-

fourths behind, hind tibia with a proximal line of pale greenish scales and a conspicuous patch of creamy-white scales at the outer third beneath. Front tarsi with lines of whitish scales beneath the first two segments, most conspicuous on the second; mid tarsi with white basal bands on the first two segments, that of the second being about two-thirds of its length; hind tarsi with sub-basal white band on the first segment, second segment entirely dark and third segment with a broad basal creamy-white band. *Wings*. Scales with metallic greenish reflections, posterior border with emargination well marked, length of wing about 5 mm.

*Pupa*. Respiratory trumpets with the opening deeper than in *M. brevipalpis* Theo. (Macfie and Ingram, 1923), the ratio of the length of the closed portion to that of the whole about 1 : 1.9. Paddles closely resembling those of *M. brevipalpis*. Macrochaetae of abdomen differing considerably from those of that species, lateral only present on fifth segment, projecting at right-angles; second segment with sub-lateral macrochaeta about equal in length to the segment behind; sub-median slightly shorter, a large trifid seta just internal to sub-median, its branches about equal in length to this seta. Third, fourth and fifth segments with sub-median about equal in length to the segments, sub-lateral considerably longer, that of the fifth extending beyond the distal border of the seventh segment; sixth segment with only sub-lateral represented, seventh and eighth segments without macrochaetae.

Type: one female bred from a larva taken from a tree-hole at Hill Station, Freetown, 29.vii.1925, by an officer of the Sanitary Department, Sierra Leone.

A second specimen bred from a larva taken from a hole in a Mango Tree at Hill Station, 11.vi.1924, differs in the amount of white on the legs. The front and mid tibiae are entirely dark-scaled and the hind tibiae have only a trace of the distal white patch. The front tarsi are without any pale scales and the middle tarsi have the white band on the second segment narrower than in the type. The pupal pelt shows considerable differences in the abdominal chaetotaxy, although it differs much more from *M. brevipalpis* in this respect; in the absence of a large series of specimens for comparison, it is impossible to say whether this represents a distinct species.



*Anopheles smithii* Theo. (1905) (Pl. VII).*A. (Feltinella) pallidopalpi* Theo. (1907).

The above synonymy which was put forward by Christophers (1924) in his 'Provisional List and Reference Catalogue of the Anophelini' has been confirmed by the examination of a large series of adults of both sexes reared from larvae collected at Mount Aureole, Freetown, by Professor Blacklock and the writer. The larvae, which are described by Blacklock and Evans in a paper published concurrently with this, are very characteristic, and show little variation. The adults, however, exhibit a striking dimorphism in the wing markings of the two sexes, the females having the pale spots so much reduced that the wings appear quite dark to the naked eye, while the wings of male specimens have well-developed *Myzomyia* spotting. The confusion to which this peculiarity has given rise is increased by the fact that in each sex the wing markings exhibit a striking degree of individual variation. A short account of the species with special reference to the wing markings is as follows:—

**FEMALE.** General colouration black; palpi with three narrow, rather obscure, pale bands; mesonotum clothed chiefly with hairs, scales being present only on the anterior promontory (Christophers, 1924) legs entirely black-scaled. *Wings.* The most extensively pale scaled specimens (Pl. VII, A) show small spots at the following points:—costa: just beyond the end of the sub-costa, at the apex and between these points; sub-costa: about mid-way between base and apex; first vein: near the base, opposite the sub-costal spot, opposite the sub-apical and (sometimes) apical costal spots; wing field: at the base of the fork cells; on the second, third, fourth and upper branch of fifth vein in the region of the cross-veins. This condition occurred in about one-third of the specimens; in others one or more of the pale spots were absent, but the suppression of some of the spots and retention of others was entirely promiscuous, and almost every possible variation of pattern was observed. In two examples ~~pale~~ scales were entirely absent and in several specimens only two ~~of~~ three obscurely pale scales were present. Spots may be reduced almost to extinction either by reduction of the number of pale scales involved or by being rather dusky so that the contrast

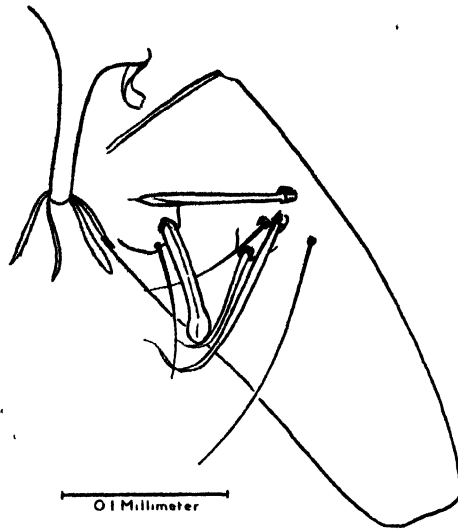
with the dark scales is not well-marked. Usually the two wings of the same specimen were of similar pattern.

Wing length: 2.8 to 3.5 mm.

**MALE.** The male is considerably lighter in appearance than the female owing to the extensive white scaling of the wings. The characters of the head, palpi, mesonotum, abdomen and legs did not show any marked variation and agreed with those of *A. pallidopalpi* as described by Theobald (1907). *Wings.* Theobald's description of the wing markings would apply to some of the specimens met with; the majority of our specimens, however, had the basal border spot involving the costa as well as the first vein, a condition which Theobald noted in one of his specimens. Typical examples with well-developed spotting (Plate VII, B) showed, in addition to the three large white spots involving the costa and first vein, a small apical spot. There were also well-marked spots in the following situations:—first vein: towards the base and just beyond the first border spot ('accessory sector spot,' Christophers); at the bases of the fork cells; at the cross-veins; on the basal half of the third vein; two extensive areas on the fifth vein and two smaller ones on its upper branch; one on the sixth.

In two exceptional cases a large white area also occurred distally on vein three. Other specimens showed reduction of some of the pale areas, the reduction, as in the female, not following any definite plan but involving sometimes one, sometimes another spot or combination of spots. The paleness of the scales also varied greatly in intensity.

**HYPOPYGIUM** (fig. 3). Christophers (1925) refers to the *Myzomyia*-like character of the hypopygium in imperfectly displayed specimens in the British Museum, labelled *smithii* and *pallidopalpi*. Our material shows that three of the five parabasal spines are very broad, the outermost being almost blade-like.



*A.M.E.*

FIG. 3. *A. smithi*. Side-piece and phallosome of male hypopygium.

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**PLATE VII**

EXPLANATION OF PLATE VII

*Anopheles smithii* Theo.

- A. Wing of female.
- B. Wing of male.

# THE MOUTH PARTS, ALIMENTARY TRACT, AND SALIVARY APPARATUS OF THE FEMALE IN *PHLEBOTOMUS PAPATASII*

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## PLATES VIII-XIV

The external morphology of the mouth parts of *Phlebotomus papatasii* has been described by Grassi (1907) and by Newstead (1911), but so far as we are aware, a detailed study of the biting, pumping and salivary apparatus has not been made for any member of the genus *Phlebotomus*.

The following study was made on *Phlebotomus papatasii*, the commonest sandfly of Palestine. The method of study adopted was observation of the mouth parts *in vivo*, dissection and serial sections, transverse, sagittal and coronal of the head.

Observation *in vivo* is the most satisfactory method of studying the action of the mouth parts. A sandfly is lightly anaesthetised with ether so that it remains motionless for about ten minutes, during which time the mouth parts are usually working actively, so that their movements can be observed under the microscope, and the contractions of the muscles of the buccal cavity can also be seen and counted. If the insects are stunned by shaking them vigorously in a test-tube, and their wings and legs removed, the movements of the external mouth parts and the contraction of the muscles of the buccal cavity will often continue up to four hours, during which period they can be conveniently studied.

Dissection of the mouth parts can be made in freshly-killed insects, but for permanent mounted preparations it is advisable to leave the insects overnight in a 5 per cent. solution of potash before dissecting.

Serial sections were made after fixation in Henning's solution or Dobell's fluid, as recommended by Hoare (1921).

There are several formulae for Henning's solution differing in the amount of nitric acid they contain; the following formula, taken from Bolles Lee (1921), was found useful for sandflies and mosquitos.

Nitric acid	...	...	...	...	...	16 parts
Chromic acid, 0.5 per cent.	...	...	...	...	...	16 parts
Saturated solution of mercuric chloride in 60 per cent. alcohol	...	...	...	...	...	24 parts
Saturated solution of picric acid in water	...	...	...	...	...	12 parts
Absolute alcohol	...	...	...	...	...	42 parts

After fixation in Henning's solution for one day, the insects were placed for two hours in a mixture of equal parts Lugol's solution and 70 per cent. alcohol; they were then passed through changes of 70 per cent. alcohol till no trace of iodine remained. Some specimens were passed through alcohol and paraffin in the usual way and others were stained in bulk, either in a saturated solution of eosin in 70 per cent. alcohol for two days, or in haematoxylin for three days, and then treated in the usual manner. After fixation in Henning's solution for twenty-four hours, sections cannot be cut less than  $20\mu$  thick. For a study of the musculature of the mouth parts and head, sections  $50\mu$  thick are the most convenient. Staining in bulk for three days in Ehrlich's haematoxylin gives the best results for histological purposes after fixation in Henning's solution.

Dobell's process is very satisfactory for histological purposes, for after fixation in Dobell's fluid, sections  $7\mu$  thick can be obtained, but the process is a lengthy one and is not necessary for the study of the muscular system.

## MOUTH PARTS

The proboscis consists of the labrum-epipharynx, hypopharynx, two mandibles, two maxillae and a labium. The length of the proboscis from the mouth to the tip of the epipharynx is  $400\mu$ . The armed parts when at rest lie unsheathed in the labium in the following manner: the labrum-epipharynx is superior and the two mandibles lie one above the other immediately between the epipharynx and hypopharynx; the maxillae lie beneath and lateral

to the hypopharynx and for the greater part of their course appear moulded to the inferior lateral aspect of the latter. It will be seen from Pl. IX, figs. 7 and 8, Pl. XIII, figs. 14 and 15, that when the parts are at rest the epipharynx and one of the mandibles form a canal, the roof of which is a groove on the inferior surface of the epipharynx, and the floor the upper surface of one of the mandibles. This canal is directly continuous with the buccal cavity and we have found it to contain *Herpetomonas* five days after feeding on an oriental sore.

The distal ends of the epipharynx, hypopharynx and mandibles lie at the extremity of the labium between the labella, and the distal ends of the maxillae lie a varying distance behind them. Owing to the disposition of the mandibles the teeth of the epipharynx and hypopharynx are never in contact when the insect is at rest.

The labrum-epipharynx is  $400\mu$  long. The labrum is a thin chitinous band which rises from the anterior superior end of the clypeus and is attached to the epipharynx first by a narrow strip of muscle and then by loose membranous tissue; it becomes fused to the epipharynx a little behind the distal end of the latter.

The epipharynx is  $400\mu$  long and  $40\mu$  at its broadest part; the middle of its lower surface is grooved by a channel which, in transverse section, is triangular, the apex of the triangle being rounded (Pl. IX, figs. 7 and 8, Pl. XIII, figs. 13-15); this channel forms the roof of the food canal during the act of feeding. The distal end of the epipharynx is pointed and toothed, each tooth being curved and pointing forwards, and its toothed margin is concave.

The hypopharynx is  $400\mu$  long and  $40\mu$  at its broadest part. It is pierced through the whole of its length by the salivary canal; its distal end is toothed, the teeth being smaller than those of the epipharynx, and the toothed margin is convex. The upper surface of the hypopharynx forms the floor of the food canal during the act of feeding.

The mandibles are  $420\mu$  long and  $30\mu$  in their broadest part, and rise from the clypeus above, behind, and lateral to the mouth. The base of the mandible is divided into two cornua, one external and one internal. Rising from the infero-lateral aspect of the clypeus is a sclerite (Pl. XI, fig. 2) which curves upwards and inwards and terminates in a free point at the side of the clypeus; the free end of this sclerite lies between the two cornua of the mandible (Pl. XI,



fig. 2) and plays an important part in regulating the movements of the latter. The cornua of the mandible are darkly pigmented and form a well-marked external feature of the cranium of *Phlebotomus papatasi*.

The mandible passes downwards and inwards for a short distance and the inner side of this portion shows a strongly-marked ridge into which the adductor muscle of the mandible is inserted. Beyond the adductor ridge the mandible turns inwards and passes straight downwards between the epipharynx and hypopharynx. The distal end of the mandible is sharply pointed and its inner margin is serrated for a distance of  $60\mu$  (Pl. XI, fig. 1). The tip of each mandible is seen at the opposite side of the proboscis distally (Pl. XIV, fig. 2).

The maxillae are composed of two parts, a blade which is extra-cranial and a long process which is intra-cranial. The blade is  $330\mu$  long and  $30\mu$  in its broadest part; at its distal end, externally, there are five or six tooth-like processes pointing backwards and gradually diminishing in size from before backwards; a part of the internal margin,  $120\mu$  long and commencing  $35\mu$  behind the distal end, is also armed with tooth-like processes which point forwards. The long process of the maxillae (Pl. XI, fig. 3) is a rod of chitin  $325\mu$  long which runs backwards along the floor of the cranium; in a cleared preparation of the head it appears lateral and inferior to the whole of the buccal cavity and a part of the pharynx; it is attached by a broad chitinous band to the floor of the cranium and the points of attachment on the two sides are connected by a thin strip of membrane (Pl. XI, fig. 3).

The labium, when at rest, is  $420\mu$  long and  $110\mu$  at its broadest part, i.e., at the level of the labella, and is, with the exception of several structures to be described later, a soft organ with membranous walls. The ventral and lateral surfaces form the mentum and the concave dorsal surface forms the labial gutter (Pl. IX, figs. 7 and 8), in which the armed parts are ensheathed when the insect is at rest.

The mentum rises from a horse-shoe shaped piece of chitin (Pl. XIV, figs. 3 and 4) which forms the anterior end of the gular region and serves as the origin of the intrinsic labial muscles. The labium itself contains, in addition to the muscles, a large amount of fat, two main tracheae and their branches and the two labial nerves which rise from the middle of the lower border of the inferior ganglion

of the brain. The cavity of the labium is connected by a wide opening with the body cavity. The two labella are articulated by a chitinous disc to the labium. A median lobe or glossa, according to Grassi's terminology, lies between the two labella. The structure of the labella and glossa can only be suitably studied in fresh preparations when the whole labium is contracting and the labella become separated and the glossa distended.

When the labium is at rest the median ends of the chitinous discs which form the base of the labella are almost in contact (Pl. XIV, fig. 3), the glossa is almost completely hidden from view and its details have therefore been overlooked in previous descriptions.

On the ventral side the median end of the base of the labellum is articulated through a small rod of chitin (Pl. XIV, fig. 1) to an elongated chitinous rod which passes inwards and backwards to unite with its fellow from the other side (Pl. XIV, figs. 1, 3 and 4), and form a support for the ventral side of the labium.

On the median half of the dorsal surface of the labella are a number of fine, closely-set lines or grooves running almost horizontally (Pl. XIV, fig. 1) which produce the appearance of a pseudo-tracheal membrane. Near the distal ends of each labellum, dorsally, are four pre-stomal teeth, three median, which are close together, and one individual tooth external and more distal than the others (Pl. XIV, fig. 1). A minute muscle is inserted into the base of each tooth.

Inside each labellum near the distal end there is a group of nerve cells.

Ventrally each labellum is divided into a proximal and a distal half by a fine chitinous line (Pl. XIV, fig. 1) which, after pursuing a horizontal course till it almost reaches the internal border of the labellum, passes backwards to join the median end of the base of the latter. On the distal half of the labellum, ventrally, are a number of long and rather stout hairs.

The glossa (Pl. XIV, figs. 1, 2 and 4) is a transparent structure divided into two by a median longitudinal line; running through the greater part of each half is a thick chitinous line which is attached proximally to the median end of the base of the labella (Pl. XIV, fig. 1); from this chitinous line others radiate and the glossa folds up along these chitinous lines when the labium returns to the resting position. Distally the dorsal surface of the glossa is finely corrugated.

The palps have been amply described by Newstead, who noted that the fourth and fifth segments are bent downwards and backwards in such a way that the palps protect the proboscis. The third segment extends slightly in front of the proboscis. In Palestine *P. papatasi* shows an interesting local variation from the same species as described by Newstead, from Malta. In Malta, Newstead found that the fourth and fifth segments are distinctly annulated; in Palestine the second and third segments are also distinctly annulated.

### THE MUSCULATURE OF THE MOUTH PARTS AND THE METHOD OF BITING

In anaesthetised specimens of freshly-killed sandflies it is seen that the greater part of the gular region moves backwards and forwards in one plane. The part of the gular region that is involved in this movement is separated by a fold of membrane from a narrow area which extends in front of the occipital foramen and is bounded latero-posteriorly by the narrow inferior openings of the two intra-cranial tunnels.

During retraction the fold between the motile part of the gular region and the narrow area in front of the occipital foramen deepens and the membranous floor of the motile portion bulges downwards.

The intra-cranial tunnels are two hollow chitinous rods (Pl. XI, fig. 4), one on each side, which are connected with the exterior, superiorly and anteriorly, by a wide funnel-shaped opening in front of the base of the first antennal segment, and inferiorly and posteriorly by a narrow opening at the level of the posterior margin of the eye. Anteriorly they are connected by transverse chitinous tubes which unite in the middle line and are produced backwards as a chitinous bar which, after a short distance, branches into two chitinous bands which fuse with the roof of the cranium (Pl. XI, fig. 4). The superior ends of the intra-cranial tunnels serve as an origin for the intra-cranial muscles to the first antennal segment.

The muscles responsible for this movement are: (1) a pair of powerful protractor muscles (Pl. XII, fig. 1) which rise, one on each side, from the roof of the clypeus and passing downwards and backwards are inserted into the posterior part of the movable

portion of the gular region ; (2) a pair of retractor muscles, one on each side, which rise partly from the floor of the cranium immediately behind the inferior opening of the intra-cranial tunnel and partly from the lower part of the intra-cranial tunnel, and are inserted into the extreme anterior end of the gular region (Pl. XII, fig. 1).

The labium, maxillae and palps are attached to the gular region and they are, therefore, carried backwards and forwards during the similar movements of the latter.

The maxillae and palps have, in addition, muscles peculiar to themselves. The maxilla is supplied by a muscle which rises from the roof of the clypeus behind the protractor of the gular region and passes downwards and forwards to be inserted into the long process of the maxilla a little behind the junction of the latter with the blade (Pl. XII, fig. 2) ; thus although receiving directly only the insertion of one muscle, the maxilla is acted upon by three muscles, owing to its attachment to the floor of the cranium, and of all the armed mouth parts the maxilla has directly and indirectly the largest and most powerful supply of muscles.

From the posterior part of the floor of the movable portion of the gular region a muscle arises which passes forwards and slightly upwards to be inserted into the first segment of the palps and acts as an elevator and abductor of the palps. During the act of feeding the palps are elevated and abducted.

The mandibles are supplied by two relatively powerful muscles (Pl. XII, fig. 2) which rise from the posterior part of the cranium laterally and inferiorly and pass forwards and slightly upwards. The internal and narrower of these two muscles (Pl. XII, fig. 2, mm.) is inserted into the adductor ridge of the mandible, and the external and broader muscle (Pl. XII, fig. 2) is inserted into the tip of the external cornu. The external muscle, shortly before its insertion into the external cornu, turns round an invagination into the side of the clypeus and passes outwards for a short distance ; this muscle abducts and rotates the mandible externally. The internal muscle pursues a straight course forwards and medianwards towards insertion and, on contraction, causes adduction and internal rotation of the mandible. When the mouth parts are in action the mandibles are rapidly abducted and adducted through a narrow angle and are at

the same time rotated, movements which can be readily followed *in vivo* under the microscope. Abduction and adduction are both limited by the wedge of chitin which lies between the two cornua, for when the mandible is abducted through a narrow angle the external cornu is pressed against the wedge of chitin and further abduction is impossible; when the mandible is adducted through a narrow angle the internal cornu is pressed against the wedge of chitin and further adduction is impossible. When the mandibles are in action it is seen that the cornua move in an arc round and in front of the wedge of chitin lying between them; the mandibles, as judged by the movements of the cornua, also undergo a limited amount of backward and forward movements owing to the elasticity of the sclerite from which they rise. During the act of biting the inner dorsal margin of the expanded labella also imposes a limit to the abduction of the two mandibles.

The epipharynx and labrum are supplied by laterally symmetrical muscles, two of which function as regulators of the diameter of the food canal during the act of feeding (Pl. XII, fig. 1).

These muscles are: (1) a muscle which arises from the commencement of the labrum and, passing downwards and slightly forwards, is inserted into the epipharynx; (2) a muscle which arises from the roof of the clypeus posteriorly and, passing obliquely downwards and forwards, is inserted into the junction of the epipharynx and roof of the buccal cavity.

A third muscle arises from the anterior part of the roof of the clypeus and, passing through the origin of the labrum, is inserted into the under surface of the latter.

We are now in a position to understand the rôles played by the various armed mouth parts in biting. The maxillae, mandibles, epipharynx and hypopharynx all act as piercing stylets, but the most important rôle is played by the maxillae, for they have the widest range of movement backwards and forwards, and directly and indirectly possess the most powerful muscle supply of all the biting parts. The mandibles, through their rapid movements of adduction, abduction and rotation, combined with the small backward and forward movements described above, penetrate and enlarge the wound in all directions.

Patton and Cragg (1913) are of the opinion that the epipharynx

and hypopharynx do not play the part of active piercing stylets in the mosquito during the act of biting, but it is doubtful whether this view holds in the case of *Phlebotomus papatasi*. We have observed two males of *P. papatasi* containing blood, one of them gorged with fresh blood actually leaving a human being; in both cases the mouth parts were characteristic of the normal male, i.e., the mandibles were absent and the maxillae unarmed. It is interesting to note that in both cases dissection showed the genitalia, external and internal, to be of the normal male type and no trace of hermaphroditism was found.

During the act of feeding the mandibles no longer interpose between the epipharynx and hypopharynx and the two latter coming into opposition form the food canal; their teeth interlock and probably act as a strainer, preventing particles of too large a size from entering the food canal.

The labium undergoes interesting changes during the act of biting. There are two sets of intrinsic longitudinal muscles in the labium, all arising from the chitinous base of the mentum. An external longitudinal muscle is formed by two bellies which arise from the outer part of the base and unite into one tendon which is inserted into the lateral margin of the union of the body of the labium and the base of the labellum (Pl. XIV, fig. 4). Since the labium is a lax structure composed mainly of soft tissues, the external longitudinal muscle causes a decrease of length and an increase of breadth of the whole labium. The internal longitudinal muscle is composed of four bellies which rise from the median part of the base of the labium and pass downwards; at the level of the base of the labella they unite to form a single tendon which passes outwards through the labella and is inserted into the distal end of the latter (Pl. XIV, fig. 4). Contraction of this muscle causes abduction and expansion of the labella. Abduction of the labella is also secured indirectly by the action of the external longitudinal muscle of the labium, for the latter, by increasing the girth of the labium, causes distention and abduction of the labella. As the labella are abducted the median lobe or glossa comes into view; the glossa expands and opens out along the chitinous rays described above in the manner of a fan opening out. When the labium returns to the normal resting position the glossa again folds up. The labium when contracted by the action

of its intrinsic muscles is up to  $120\mu$  shorter than the resting labium and it is still further shortened by pressure against the skin; the biting parts projecting beyond the labella have thus ample room for piercing the skin and reaching the blood capillaries.

When the armed mouth parts are in action they lie on the expanded glossa and the median dorsal margin of the labella forms the walls of a groove, guides the mouth parts and restricts the range of their action laterally.

The above description of the movements of the mouth parts of *Phlebotomus papatasi* applies in general principles to the movements of the mouth parts of *P. minutus* and *P. perniciosus* and they may, therefore, be considered as characteristic of the genus *Phlebotomus*.

*Phlebotomus papatasi* usually bites during the night and early morning but occasionally, under natural conditions, also bites during the day; and in the laboratory specimens which have been starved several days frequently bite and feed readily by day. In our experience about 60 per cent. of specimens under laboratory conditions refuse to feed under any circumstances and die of starvation. Specimens in which the eggs are ripe or nearly ripe usually refuse to feed. *P. papatasi* often bites several times before feeding and we have observed one specimen bite seven times on an area of skin half-an-inch in diameter before feeding. There is an interval of fifteen to thirty seconds between the commencement of the act of biting and the entrance of blood into the buccal cavity; aetiologically this interval is important for it gives an opportunity for parasites in the proboscis to enter the wound. When blood is already flowing into the buccal cavity the negative pressure caused by the muscles of the latter would tend to prevent parasites from the food canal entering the wound.

The buccal cavity is formed by the union of the continuation backwards into the cranium of the epipharynx and hypopharynx; it is composed of three chitinous plates, an inferior one which forms the floor of the cavity and is strongly chitinised, and two lateral plates which meet in the mid-line and are feebly chitinised except at their lateral margins. The continuation of the hypopharynx inside the cranium splits into two laminae, a superior one which is strongly chitinised and convex ventrally, and which forms the floor of the buccal cavity, and an inferior one which is continuous posteriorly

with the inferior part of the common salivary duct (Pl. X, fig. 2). The salivary pump lies between these two laminae.

It is difficult to give an exact verbal description of the shape in transverse section of the buccal cavity, for this differs at various points and can be best appreciated from the figures of transverse sections of the clypeus at various levels (Pl. IX, fig. 6, Pl. XIII, figs. 8-12).

It will be seen that the lumen of the buccal cavity when at rest is very narrow and of a peculiar shape, roughly triangular with the base of the triangle thick and slightly convex downwards, and the sides of the triangle extremely concave inwards.

The lateral part of the backward continuation of the epipharynx is, in marked contrast to the median portion, strongly chitinated, and forms a strong bar of chitin which is fused to the lateral margin of the floor of the buccal cavity. This bar passes upwards and backwards (the general direction of the buccal cavity) and splits into two bars, one inferior and one superior. The inferior bar (Pl. X, figs. 2 and 3) proceeds backwards and then turns downwards and passes below the floor of the buccal cavity, and meeting its fellow from the opposite side forms an arch convex backwards. This arch forms a support for the buccal cavity and during the whole of its course (Pl. XII, fig. 3) serves as an origin for a relatively large and powerful salivary muscle which, converging from all points of the arch, passes downwards and forwards to be inserted into the salivary pump (Pl. VIII, fig. 1, Pl. XII, fig. 3). The upper bar passes upwards and backwards and terminates in a cornu on each side, the two cornua being united by two cross-pieces, one anterior and the other posterior (Pl. X, fig. 3). The connecting tube between the buccal cavity and the pharynx lies below the two cross-pieces.

The buccal cavity is supplied by a large group of muscles which rise from the roof of the clypeus near the middle line and are inserted into the two chitinous plates which form the roof of the buccal cavity. The general direction of these muscles is downwards and forwards, but a few longer than the others pass downwards and backwards from the roof of the clypeus and are inserted into the most posterior part of the buccal cavity (Pl. XII, fig. 4).

The action of the muscles of the buccal cavity can be studied *in vivo*, when they are seen to contract up to one hundred and twenty times a minute; they act as pumping organs, for by creating a



negative pressure they pump blood into the buccal cavity through the food canal. When the muscles contract the two superior chitinous plates are pulled upwards and outwards and the buccal cavity is thus dilated (Pl. XIII, fig. 10).

Immediately behind the buccal cavity and lying below the two cross-pieces is a small chitinous tube  $30\mu$  long which joins the buccal cavity to the pharynx. This tube is surrounded by a sphincter muscle which regulates the flow of blood from the buccal cavity into the pharynx (Pl. VIII, fig. 1, Pl. XI, fig. 4),

The pharynx is  $210\mu$  long and  $63\mu$  in its broadest part; it is broad posteriorly and narrow anteriorly. It is composed of three chitinous plates, one superior and horizontal and two lateral. The transverse section of the lumen of the pharynx varies at different levels but it is roughly triangular in shape, the base of the triangle being superior and all three sides concave internally. Posteriorly ridges are seen on the wall of the pharynx; these are the optical expression of internal teeth which extend for a distance of  $80\mu$  along the lateral walls and for a slightly shorter distance along the superior wall. The foremost teeth are small and point backwards and the remainder are vertical (Pl. XII, fig. 4).

The pharynx, except for a small portion posteriorly and another anteriorly, lies inside the brain.

The pharynx is supplied by the following bilaterally symmetrical muscles:

(1) A dorsal anterior group of muscles which rise from the roof of the cranium immediately behind the *clvpeus* and pass downwards to be inserted into the superior plate of the pharynx. The anterior fibres pass vertically downwards and the posterior ones obliquely downwards and backwards between the superior ganglion of the brain and the pharynx (Pl. VIII, fig. 1, Pl. XII, fig. 4).

(2) A dorsal posterior group of muscles which rise from the roof of the cranium above and in front of the occipital foramen and pass downwards to be inserted into the superior plates of the pharynx. The anterior fibres of this group pass obliquely forwards and downwards between the superior ganglion of the brain and the pharynx (Pl. VIII, figs. 1 and 2, Pl. XII, fig. 4).

(3) A powerful group of muscles which rise from the infero-lateral aspect of the cranium posteriorly and pass upwards and forwards to

be inserted into the inferior plates of the pharynx. The anterior fibres of this group pass obliquely upwards and forwards between the pharynx and the inferior ganglion.

Although the greater part of the pharynx lies inside the brain, yet owing to the peculiar direction of the muscles almost the whole surface of the pharynx serves as an insertion for dilator muscles.

The function of the above-described muscles is to pump blood from the buccal cavity into the pharynx, the short tube lying between the pharynx and buccal cavity acting as a regulator of the flow of blood.

The oesophagus is a short tube  $80\mu$  long, as measured from the posterior opening of the pharynx to the commencement of the midgut. It is attached for a considerable distance to the sides of the pharynx and thus a pouch is formed between the external wall of the pharynx and the oesophagus (Text-fig. 1). This pouch has been found to contain *Herpetomonas*. The wall of the oesophagus is lined by a single layer of epithelium which lies on a basal membrane; the interior surface of the epithelium is covered with a very fine layer of chitin.

The oesophageal diverticulum lies ventral to the midgut; it opens into the oesophagus at a varying distance from the posterior end of the latter; exceptionally it opens into the pharynx together with the oesophagus. The diverticulum is composed of two very fine layers of muscle fibres, one longitudinal and internal and the other circular and external; internally it is lined with flat epithelium covered by a thin layer of chitin. There is a very narrow sphincter at the junction of the oesophagus and diverticulum. In freshly dissected insects the diverticulum is usually seen to be undergoing peristaltic movements towards the oesophagus. Unlike the oesophageal diverticulum of mosquitos the diverticulum of *P. papatasi* seldom contains air bubbles. Out of four thousand sandflies examined only one instance was observed of an air bubble in the diverticulum and this in spite of the fact that the midgut often contains air bubbles.

Waterston (1922) states that blood can be seen in the oesophageal diverticulum for about forty-eight hours after a meal, while Patton and Cragg (1913) state that the oesophageal diverticulum is filled with blood immediately after a feed but is empty several hours later. In our experience, based on a dissection of four thousand sandflies, it is unusual to find blood or bloodstained fluid in the

oesophageal diverticulum at any time, and in the few cases where blood is found it is present only in negligible quantities as compared with the amount found in the midgut. Even when the insect is fully gorged and the stomach distended to its fullest capacity, the

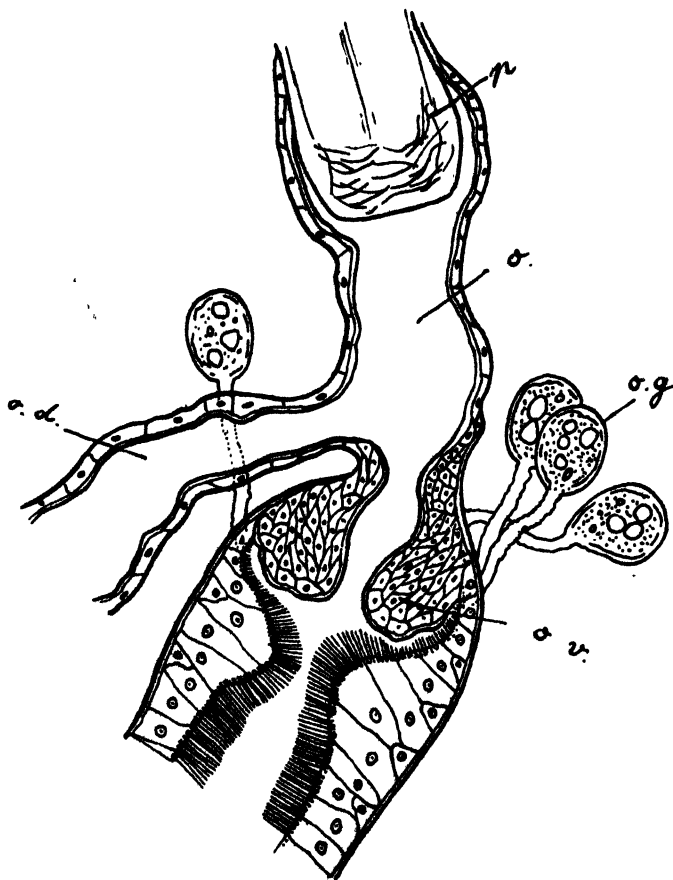


FIG. 1 (diagrammatic). *p.*—pharynx; *o.*—oesophagus; *o.d.*—oesophageal diverticulum; *o.g.*—oesophageal glands; *o.v.*—oesophageal valve.

Note rods projecting from the epithelial cells of the cardia.

Note pouch between the sides of the cardia and the valve.

oesophageal diverticulum contains very few red cells or none at all. Out of twenty-five sandflies killed immediately after a feed four contained red cells in the oesophageal diverticulum, but only in small quantities. In insects killed a few days after a feed

the oesophageal diverticulum is usually found distended with perfectly clear fluid. This fluid is later either absorbed through the thin walls of the diverticulum or is passed into the midgut. In specimens kept after feeding until the midgut is empty, the oesophageal diverticulum is usually also empty. It appears that the oesophageal diverticulum may function as a reservoir, but only for fluids and not for red cells.

There are four small elliptical oesophageal glands  $23\mu$  long and  $18\mu$  broad, which contain large yellow granules; they lie round the commencement of the midgut and each gland opens by a minute duct into the pouch between the oesophageal valve and the midgut (Text-fig. 1).

The midgut consists of two parts which differ histologically. The upper part or cardia is tubular and lies in the thorax; it is lined by a single layer of columnar epithelium of peculiar structure; near the internal surface of each cell there are a number of fine granules and from each granule a thin transparent rod projects into the lumen of the gut (Text-fig. 1). The rods somewhat resemble cilia but they are not motile or contractile; they are closely packed and in fresh undamaged preparations they are not very evident, but if the wall of the gut is broken by pressure and individual cells set free, their true nature can be readily determined. Each rod is  $7.5\mu$  to  $10.5\mu$  long. Lying between the epithelial cells near their base there are a number of small interstitial cells.

The oesophageal valve lies at the superior end of the cardia and consists of a downward projection of the oesophagus into the cardia; between this projection and the wall of the midgut there is a small pouch (Text-fig. 1 and Pl. VIII, fig. 2). The posterior surface of the pouch is lined with epithelium characteristic of the cardia.

The structure of the cardia bears an interesting relation to the development of *Herpetomonas* in sandflies. Christophers, Shortt and Barraud (1925) found large numbers of *Herpetomonas* attached to the epithelium of the upper part of the midgut in a number of specimens of *Phlebotomus argentipes* fed on a case of Kala-azar, and the authors have recorded a natural infection of *Phlebotomus papatasi* with *Herpetomonas* in which the parasites were attached to the posterior surface of the oesophageal valve. The authors, in a series of experiments in which one hundred and fifty-five specimens

of *P. papatasii* were fed on oriental sores, found that in nine out of sixteen positive cases flagellates attached themselves to the wall of the cardia, in some cases as early as the third day. The parasites can be seen boring into the epithelium with their flagella, and when they are completely attached their flagella lie entangled among the rods projecting from the epithelial cells and even reach into the cytoplasm. Division of the flagellates takes place mainly in the cardia, particularly in the anterior end which is found in artificial and in some cases in natural infections, to be completely choked up by a mass of flagellates.

The cardia is not distensible, in marked contrast to the second part of the midgut, i.e., the stomach.

The stomach lies in the abdomen and its condition depends on the amount of blood contained and the time from the last feed; it is lined by a single layer of epithelium which does not contain the rods characteristic of the cardia.

The stomach is very distensible and accommodates itself to the relatively enormous feeds of *P. papatasii*. An average female of *P. papatasii* weighs 0.3 milligrams and, after a full feed, weighs 0.4 milligrams. (It is interesting to note that *P. papatasii* never passes blood per rectum during the act of feeding.)

Digestion is a relatively slow process. It is not uncommon to find erythrocytes in a good state of preservation four days after a feed, and in one instance we observed unimpaired erythrocytes eight days after a feed. Haemolysis does not take place as a rule till the third or fourth day after a feed.

A day or two after feeding the distention of the stomach is diminished and the oesophageal diverticulum is filled with a colourless fluid, and during this period no blood is passed in the faeces nor are erythrocytes destroyed in the stomach. Unaltered haemoglobin is never found in the epithelial cells of the stomach but it is passed in the faeces. The above observations tend to show that the essential food element in the blood is the plasma and not the red cells and this is further supported by the fact that sandflies will feed when the red cells from the previous feed are still present in the stomach.

The epithelium of the midgut contains a powerful anticoagulin. In five experiments two small scratches were made close together on

human skin ; the empty midgut of *P. papatasii* was dissected out and rubbed into one scratch and the other was left untreated. Blood oozed from the scratch into which the midgut was rubbed for a considerably longer time, in one instance three-quarters-of-an-hour, than from the other.

The red cells do not come in direct contact with the epithelium of the stomach, for in *P. papatasii* (also in *P. minutus* and *P. perniciosus*) a very definite peritrophic membrane is produced. The peritrophic membrane is readily seen in specimens killed a day or two after a feed ; it is a thin white amorphous structure which contains the mass of red cells. Anteriorly it extends into the cardia and posteriorly into the hindgut. The peritrophic membrane may be likened to a sealed tube closed anteriorly and posteriorly.

The Malpighian tubes rise near the posterior end of the stomach by two common ducts, as described and figured by Newstead (1911). Near their origin the ducts divide into two tubes each about  $1,300\mu$  long and  $24\mu$  thick ; these extend downwards almost to the end of the abdomen and then curve backwards, their distal extremities lying in the upper part of the abdomen near the thorax.

Immediately behind the origin of the Malpighian tubes there is a ring of muscles which marks off the midgut from the hindgut and serves as a point of origin for the peristaltic movements of the latter. The wall of the hindgut consists of two layers of muscles, one longitudinal and external and the other oblique and internal. The lumen is lined by a single layer of cubical epithelium, the inner aspect of which is covered with an exceedingly thin glistening chitinous layer. In the posterior part of the hindgut are two large rectal papillae  $30\mu$  long and  $20\mu$  in their widest part ; these are composed of large polygonal cells with a small round nucleus. The rectal papillae are richly supplied with tracheae.

### THE SALIVARY APPARATUS

The salivary apparatus consists of the salivary glands, salivary ducts, salivary pump and the salivary channel through the hypopharynx.

The salivary glands lie one on each side in the uppermost ventral part of the thorax. They are hollow, almost spherical organs lined

with a single layer of columnar epithelium which rests on a basal membrane. A fully-distended salivary gland may reach the size of  $180\mu$  long by  $140\mu$  wide. Immediately after a feed the salivary glands are small and the epithelium thin. If a series of sandflies is dissected at various times after a feed it is seen that the epithelial cells become progressively larger and filled with granules. After a time cells are found free in the lumen of the gland, which contains, in addition, fine granules secreted by the epithelium. The free cells degenerate and break up into refractile granules much larger than those secreted by the epithelium. Three or four days after a feed the gland is distended with secretory granules and the products of degeneration of liberated cells and the epithelium lining the gland is thinned by pressure (Text-fig. 2, *a-e*). It will be seen from the above description that the saliva is composed of the products of liberated cells which degenerate in the lumen and of the secretion of the cells lining the lumen of the gland. Generally there is a parallelism between the condition of the salivary glands and the condition of the stomach.

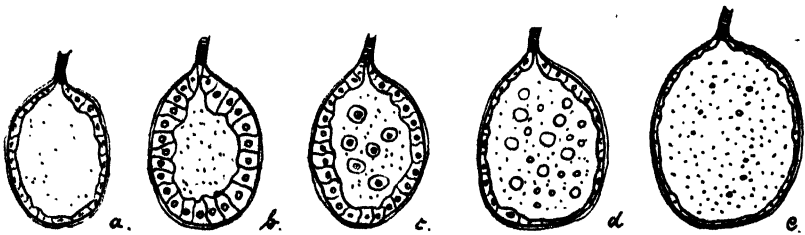


FIG. 2 (diagrammatic). Salivary glands in various stages. *a*.—immediately after a feed; *b-e*.—subsequent stages.

Note in *c*. free cells in the lumen.

Sandflies which had been kept in the laboratory for four days without food, so that digestion was well advanced and the salivary glands large, were dissected. Twenty salivary glands were placed in 20 cmm. neutral distilled water, the glands were broken up with fine needles and the resulting emulsion transferred to a capillary tube of narrow bore into which a minute amount of phenol red was drawn. The emulsion showed a faint alkaline reaction.

A series of glands were emulsified in 20 cmm. saline and various

amounts of blood were added. The mixtures were drawn into fine capillary tubes and the coagulation time compared with controls containing the same amount of saline and blood. It was found that an emulsion of eight distended salivary glands in 20 cmm. saline delayed coagulation of 2 cmm. human blood for fifteen minutes, and an emulsion of twelve salivary glands delayed the coagulation of 2 cmm. human blood for thirty minutes. (Controls showed complete coagulation in nine minutes.) Since, in nature, the contents of two salivary glands are used for not more than 0.1 cmm. blood, as compared with 0.5 cmm. and 0.3 cmm. in the above experiment, it follows that the saliva functions as an anticoagulant during the act of biting.

Emulsions of ten and twenty salivary glands in 20 cmm. of a 1 per cent. solution of sodium citrate in saline were mixed with 5 cmm. blood and the mixture drawn up in fine capillary tubes and observed under the microscope; no haemolysis and no agglutination took place during an observation period of six hours.

The salivary ducts are annulated tubes with thin chitinous walls  $140\mu$  long and a lumen  $7.5\mu$  wide (strikingly wider than the lumen of the salivary ducts of mosquitos which are  $2.5\mu$  wide). They pass into the head and converge to the middle line where they unite to form the common salivary duct. The common salivary duct is  $190\mu$  long and  $11\mu$  wide and has the same structure as the salivary ducts; it passes in the middle of the head underneath the inferior ganglion of the brain and opens into the salivary pump. The inferior wall of the common salivary duct shortly before its entrance into the salivary pump joins the inferior lamina of the hypopharynx.

The salivary pump is elliptical,  $100\mu$  long by  $45\mu$  wide (Pl. X, fig. 2, and Text-fig. 3). Its walls are formed of thick chitin and are traversed interiorly by strongly-marked circular ridges. A little in front of the entrance of the common salivary duct the floor of the salivary pump contains a small yellow elevation from which a number of minute teeth project into the lumen of the pump. Anteriorly the lumen of the salivary pump is continuous with the salivary canal which pierces the hypopharynx.

We have to thank Mr. M. Ber, of Jerusalem, for collecting a large number of sandflies and for being the subject of numerous experiments.



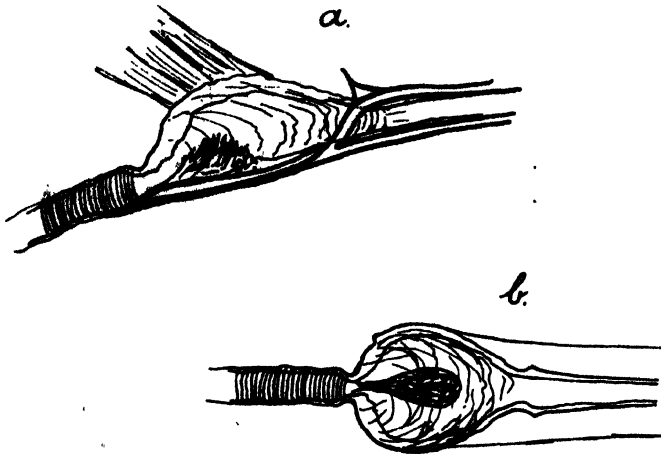


FIG. 3 (diagrammatic). Salivary pump. *a.*—side view; *b.*—dorsal view.

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### ADDENDUM

There are usually five prestomal teeth on each labellum, three set close together as described and figured above and two more distal than the others.

Since the above paper was written we have observed one instance of *P. papatasii* passing a minute amount of fluid per anum during the act of feeding but this is very exceptional and therefore not of aetiological importance.

(According to Parrot (1922) *P. minutus* var. *africanus* passes fluid per anum during feeding.)

Parrot (1922) also observed an interval between biting and the entrance of blood into the insect (temps préparatoire) ; the interval noted was three to six minutes in *P. minutus* var. *africanus* and a half to one minute in *P. papatasii*.

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15 Jan., 1926

## EXPLANATION OF PLATE VIII

Fig. 1. Sagittal section through the middle of the head of  
*P. papatasi*. × 100.

*b.m.*—Muscles of the buccal cavity.

*s.g.*—Superior ganglion of the brain.

*i.g.*—Inferior ganglion of the brain.

*c.s.d.*—Common salivary duct.

*s.m.*—Salivary muscle inserted into salivary pump.

*h.*—Hypopharynx.

*l.e.*—Labrum-epipharynx.

Fig. 2. Sagittal section through pharynx and oesophageal valve.  
× 130.

*o.*—Oesophagus.

*o.d.*—Oesophageal diverticulum.

*o.v.*—Oesophageal valve.

*p.t.*—Pharyngeal teeth.

Fig. 3. Connecting tube between pharynx and buccal cavity.  
× 150.

*c.t.*—Connecting tube.

*s.*—Sphincter muscle.



FIG. 1





PLATE IX

## EXPLANATION OF PLATE IX

Transverse sections through the head and the proboscis.

(All figures  $\times 300$ .)

Fig. 1. Pharynx at its commencement.

Fig. 2. Pharynx showing teeth.

Fig. 3. Pharynx : the two lateral plates only are toothed.

Fig. 4. Pharynx immediately before entering the brain.

Fig. 5. Pharynx in the middle of the brain.

Fig. 6. Section through the clypeus.

*s.p.*—Salivary pump.

*b.c.*—Buccal cavity. Floor and sides of the buccal cavity are thickly chitinised ; the roof is thinly chitinised.

Figs. 7 and 8. Sections through the proboscis.

The sections are arranged progressively from behind forwards.



FIG. 1



FIG. 2



FIG. 3



FIG. 4



FIG. 5



FIG. 6







PLATE X

## EXPLANATION OF PLATE X

Fig. 1. Salivary glands.

Fig. 2. Lateral view of buccal cavity.

*c.s.d.*—Common salivary duct.

*s.p.*—Salivary pump.

*el.*—Elevation on the floor of the salivary pump from which minute teeth project.

Fig. 3. Dorsal view of the pharynx and the buccal cavity.

(All figures  $\times 225$ .)

*b.c.*—Buccal cavity.

*p.*—Pharynx.

*i.b.*—Chitinous arch beneath the buccal cavity from which the salivary muscle rises.

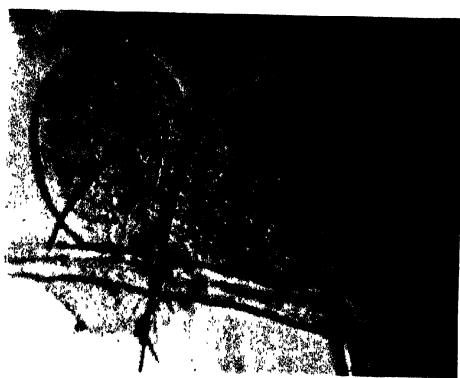


FIG. 1



FIG. 2

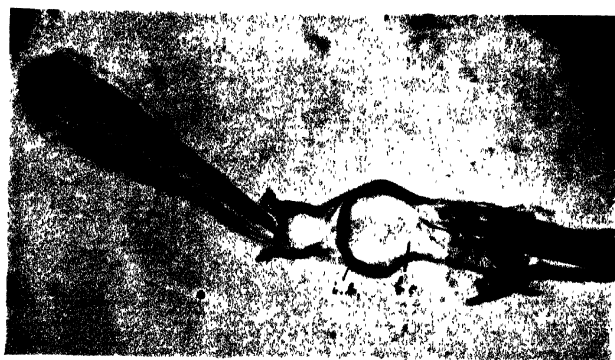


FIG. 3





## EXPLANATION OF PLATE XI

## Fig. 1. Mandible.

- e.c.*—External cornu.
- ab* .—Abductor tendon of the mandible.
- adc.*—Adductor tendon of the mandible.
- i.c.*—Internal cornu.
- r.*—Ridge into which the adductor tendon is inserted.

## Fig. 2. Origin of the mandible.

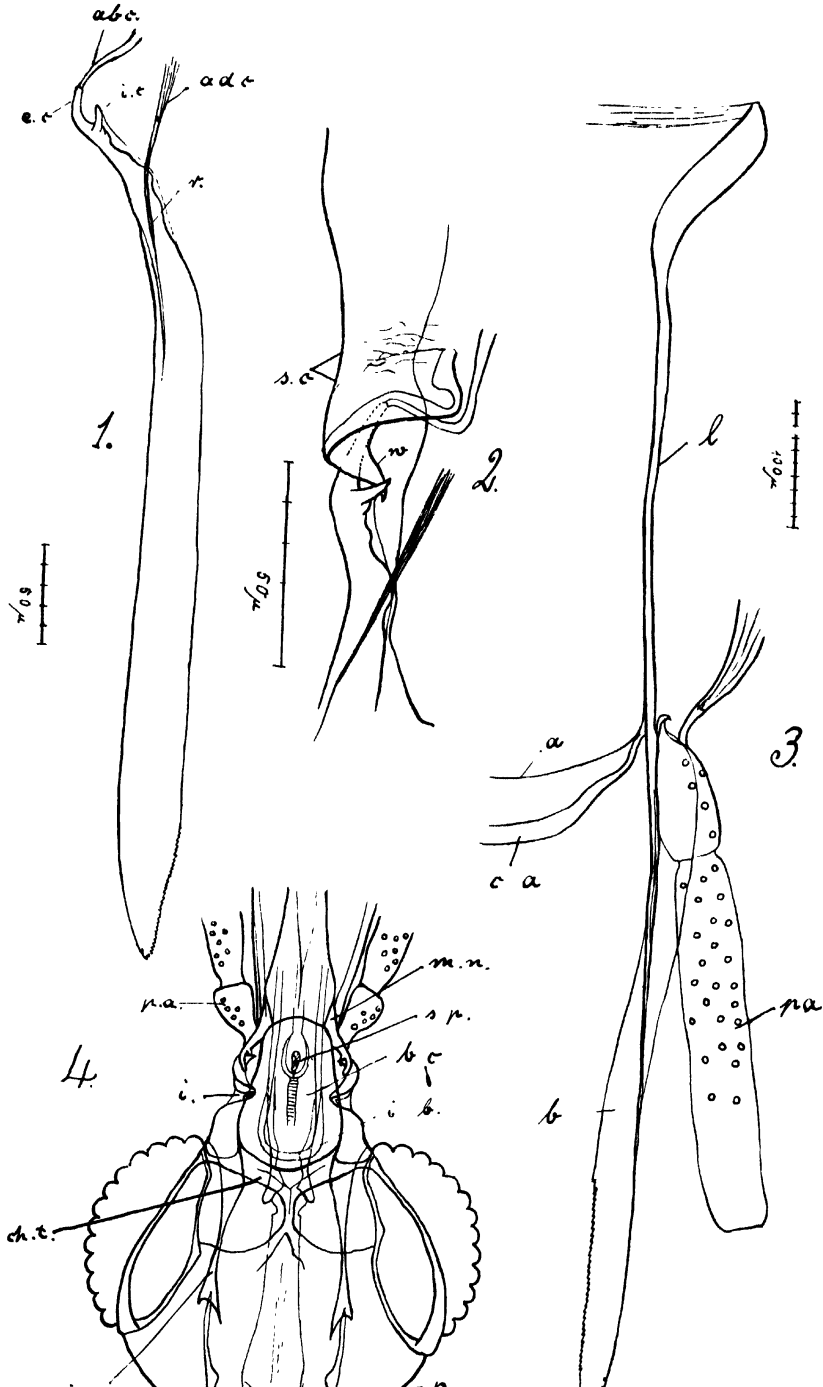
- sc.*—Sclerite from which the mandible arises.
- w.*—Wedge of chitin between the two cornua.

## Fig. 3. Maxilla.

- l.*—Long process (intracranial).
- b.*—Blade.
- a.*—Anterior margin of the clypeus.
- c.a.*—Chitinous arch at anterior part of the gular region from which the mentum arises.
- pa.*—Palp.

## Fig. 4. The head as seen in a cleared preparation from above.

- i.c.t.*—Intracranial tunnel.
- ch.t.*—Chitinous tubes uniting the intracranial tunnels anteriorly.
- p.*—Pharynx.
- b.c.*—Buccal cavity.
- i.b.*—Chitinous arch beneath the buccal cavity.
- s.p.*—Salivary pump.
- r.*—Ridge in front of the occipital foramen.
- i.*—Invagination into the sides of the clypeus round which the abductor tendon of the mandible turns.
- mn.*—Mandibles.
- pa.*—Palp.







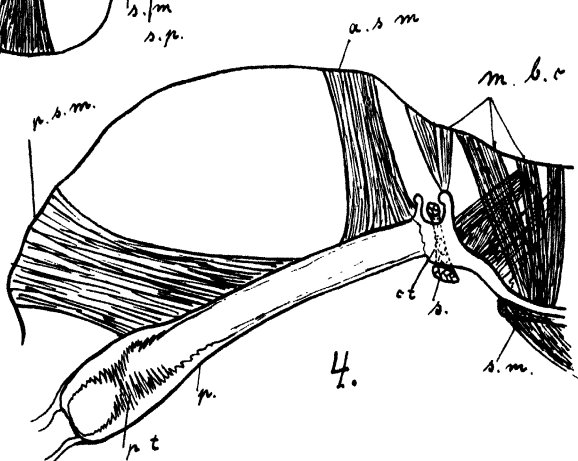
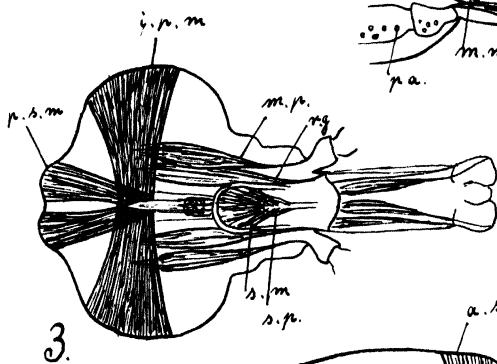
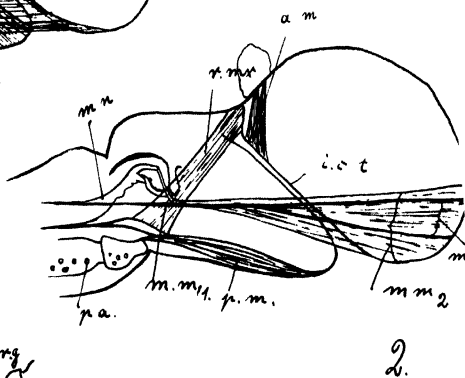
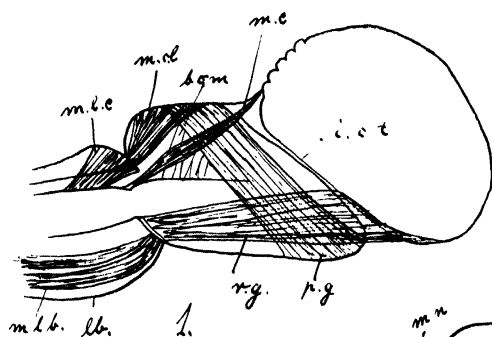
**PLATE XII**

## EXPLANATION OF PLATE XII

## The muscles of the head (semi-diagrammatic).

- Fig. 1. *p.g.*—Protractor muscle of the movable part of the gular region.  
*r.g.*—Retractor muscle of the movable part of the gular region.  
*b.c.m.*—Muscles to the buccal cavity.  
*m.l.e.*—Muscles from the labrum to the epipharynx.  
*m.c.l.*—Muscles from the clypeus to the labrum inserted into the under surface of the labrum.  
*m.e.*—Muscle from the posterior part of the roof of the clypeus inserted into the epipharynx at the junction of the latter with the roof of the buccal cavity.  
*i.c.t.*—Intracranial tunnels.  
*l.b.*—Labium.  
*m.lb.*—Intrinsic muscles of the labium.
- Fig. 2. *r.mx.*—Retractor muscle of the maxilla.  
*m.m.<sub>1</sub>*—Adductor muscle of the mandible.  
*m.m.<sub>2</sub>*—Abductor muscle of the mandible.  
*p.m.*—Palpal muscle.  
*mn.*—Mandible.  
*pa.*—Palp.  
*a.m.*—Antennal muscles.
- Fig. 3. *p.s.m.*—Posterior superior pharyngeal muscles.  
*i.p.m.*—Inferior pharyngeal muscles.  
*m.p.*—Palpal muscle.  
*r.g.*—Retractor muscle of the gular region.  
*s.m.*—Salivary muscle.  
*s.p.*—Salivary pump.
- Fig. 4. *p.*—Pharynx.  
*p.t.*—Pharyngeal teeth.  
*a.s.m.*—Anterior superior pharyngeal muscles.  
*p.s.m.*—Posterior superior pharyngeal muscles.  
*c.t.*—Connecting tube between the buccal cavity and the pharynx.  
*s.*—Sphincter muscle round the connecting tube.  
*m.b.c.*—Muscles of the buccal cavity.  
*s.m.*—Salivary muscle.

In figs. 1 and 2 the inferior opening of the intra-cranial tunnels marks the posterior limit of the movable part of the gular region.





**PLATE XIII**

## EXPLANATION OF PLATE XIII

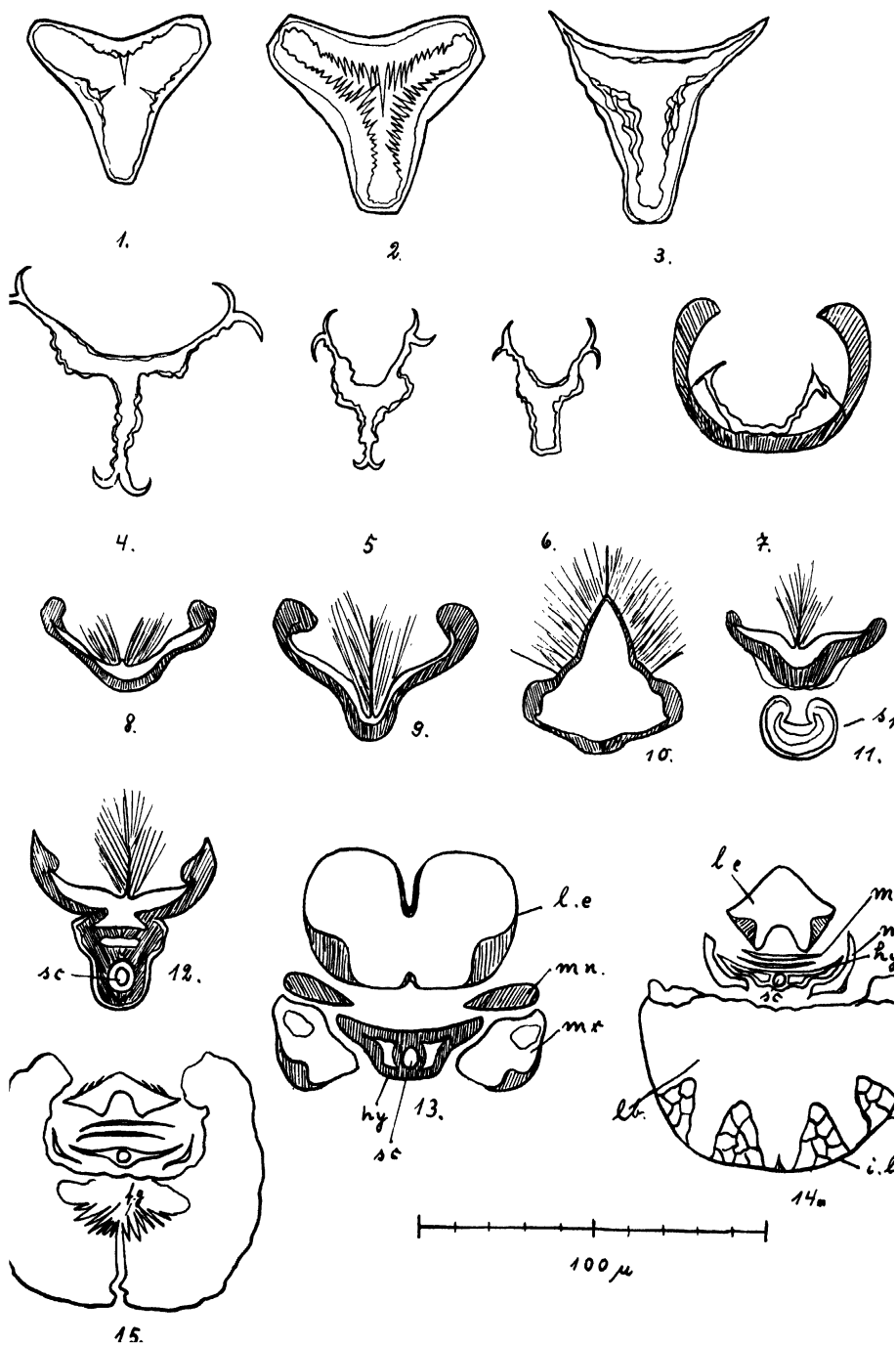
Transverse sections through pharynx, buccal cavity and proboscis at various levels from behind forwards.

(Camera-lucida drawings.)

- Figs. 1 to 4. Pharynx before it enters the brain.  
 Fig. 5. Pharynx in middle of brain.  
 Fig. 6. Anterior end of pharynx.  
 Fig. 7. Connecting tube between pharynx and buccal cavity.  
 Figs. 8 to 9. Buccal cavity.  
 Fig. 10. Buccal cavity dilated.  
 Fig. 11. Buccal cavity and salivary pump (*s.p.*).  
 Fig. 12. Buccal cavity in front of salivary pump.  
           *s.c.*—Salivary canal.  
 Fig. 13. Commencement of proboscis.  
           *l.e.*—Labrum-epipharynx.  
           *mn.*—Mandible.  
           *mx.*—Maxilla.  
           *hy.*—Hypopharynx.  
           *s.c.*—Salivary canal.

The mandibles do not yet interpose completely between the epipharynx and hypopharynx.

- Fig. 14. Section through the proboscis.  
           *lb.*—Labium.  
           *e.l.m.*—External longitudinal muscle of the labium.  
           *i.l.m.*—Internal longitudinal muscle of the labium.  
 Fig. 15. Section through the proboscis near the tip.  
           *f.g.*—Folds of glossa.









## EXPLANATION OF PLATE XIV

Fig. 1. Labella.

*A* Ventral side.

*gl.*—Glossa. Note radiation from the chitinous line along the surface of the glossa. The glossa folds and opens along these rays.

*B* Dorsal side. Note the fine lines on the labellum producing the appearance of a pseudotracheal membrane.

*p.s.t.*—Prestomal teeth.

(Camera-lucida drawing.)

Fig. 2. Dorsal view of the mouth parts at rest.

*lbl.*—Labellum.

*gl.*—Glossa.

*l.e.*—Labrum-epipharynx.

*mn.l.*—Left mandible.

*r.mn.*—Right mandible.

*mx.*—Maxilla.

The hypopharynx lies below the mandible and is not in view.

(Camera-lucida drawing.)

Fig. 3. Labium. Ventral side.

Fig. 4. Muscles of the labium.

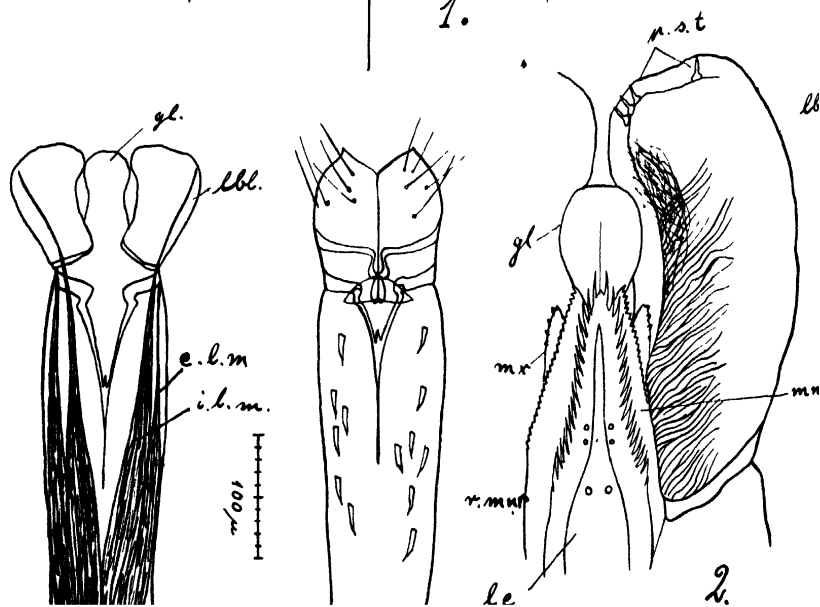
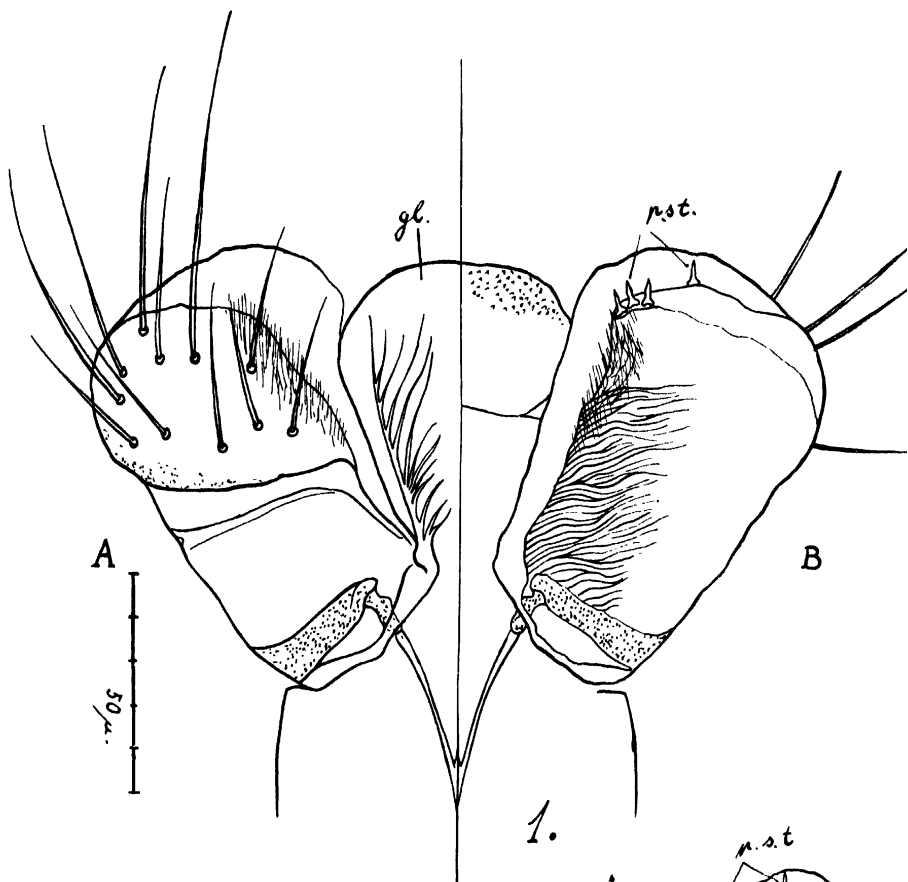
*lbl.*—Labellum.

*gl.*—Glossa.

*e.l.m.*—External longitudinal muscle.

*i.l.m.*—Internal longitudinal muscle.

(Semi-diagrammatic.)





# A CASE OF POROCEPHALOSIS

BY

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(Received for publication, 17 December, 1925)

## PLATE XV

The following case is of interest because of the rarity of the condition ; the parasite has not been previously reported from the Anglo-Egyptian Sudan.

Patient was a man aged 36 years (Zehei Bakheit), and lived some thirty miles from Khartoum ; he was a Sudanese.

He died in the Khartoum Civil Hospital on the 11th October, 1925.

## POST-MORTEM FINDINGS

At the post-mortem examination, a condition of advanced tuberculosis of the lungs with pleural effusion was found. The tubercular nature of the lesion was later confirmed by microscopical sections which showed a condition of typical broncho-pneumonic tubercle.

There were several encysted larvae of *Porocephalus armillatus* in the surface of the liver (Pl. XV, fig. 1) and also eight similar cysts in the submucosa of the duodenum.

No free nymphae were noticed in the peritoneal cavity, and no encysted larvae were found in the lungs.

## PATHOLOGY

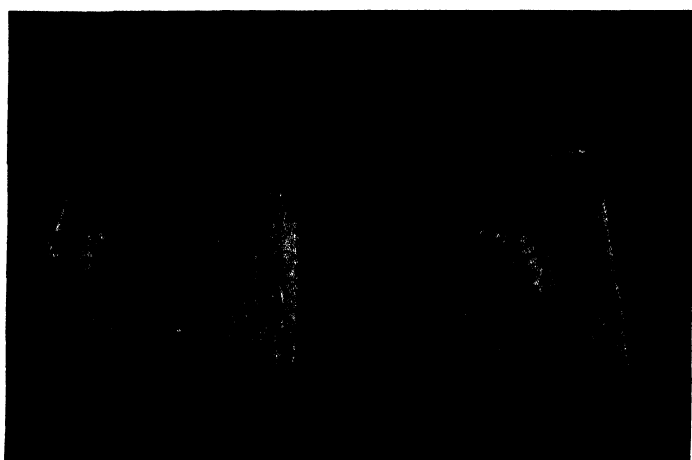
The parasite was certainly not the immediate cause of death ; the possibility that the destructive action of the parasite in the lungs predisposed to the development of the tubercular infection was considered, although no encysted larvae were found in these organs.

KHARTOUM,

3rd December, 1925.

## EXPLANATION OF PLATE XV

- Fig. 1. Photograph of larvae in surface of liver (natural size).  
The larva consists of sixteen segments and is  $1\frac{1}{2}$  cms.  
long, by 3 mm. diameter (cross section).
- Fig. 2. (Enlargement). Shows larva curled up in its transparent  
chitinous capsule, with its head towards the centre of  
the circle.







## MISCELLANEA

### *PLASMODIUM REICHENOWI*

BLACKLOCK AND ADLER, 1924

This name was given by Blacklock and Adler to a crescent-forming parasite of the chimpanzee found by them in Sierra Leone ; they concluded as the result of cross infection experiments that it was not identical with *P. falciparum*. From a personal communication of Dr. Swellengrebel to Dr. Bagshawe, Director of the Tropical Diseases Bureau, it appears that Swellengrebel and Ihle have previously applied the name *Laverania reichenowi* to this parasite in 1922, on page 12 of 'Sluiter, Swellengrebel and Ihle. De dierlijke parasieten van den mensch en van onze huis dieren, 3d. Ed. Amsterdam, 1922. Scheltema and Holkema.'

B. BLACKLOCK.



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# ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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Articles for publication should not exceed twenty-five pages of the *Annals*, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to:—The Editors, School of Tropical Medicine, The University, Liverpool.

Illustrations for text figures or charts should be drawn clearly and firmly in Indian ink, if possible on Bristol board. N.B.—*Blue or other coloured* ruling in squares or lines cannot be reproduced.

All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—‘According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.’ The references should be collected in alphabetical order of authors’ surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). ‘The spleen in malaria.’ *Annals of Nosology*, Vol. XX, pp. 20-25.

SMITH, J. (1900). ‘Enlargement of the spleen in malaria.’ *Journal of Pathometry*, Vol. I, pp. 1-20.

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## ERRATA

Vol. XX. No. 1.

Page 114. For 'In anaesthetised specimens of freshly killed sandflies' read 'In anaesthetised specimens or freshly killed sandflies.'

Page 127. For 'The salivary pump is elliptical,  $100\mu$  long by  $45\mu$  wide' read 'The salivary pump is elliptical,  $50\mu$  long by  $35\mu$  wide.'

Page 130. For 'Fig. 1.  $\times 100$ ' read 'Fig. 1.  $\times 175$ .' For 'Fig. 2.  $\times 130$ ' read 'Fig. 2.  $\times 200$ .'

# SUSCEPTIBILITY AND RESISTANCE TO TRYPANOSOME INFECTIONS

## I

### ATTEMPTS AT IMMUNIZATION WITH DEAD AND ATTENUATED TRYPANOSOMES

BY

I. J. KLIGLER

AND

I. WEITZMAN

*(From the Malaria Research Unit, Haifa, Palestine)*

*(Received for publication 14 December, 1925)*

Most observers who have had occasion to study malaria have been impressed by the fact that a recovery from an infection confers a certain degree of resistance. We have noted repeatedly that in communities in which malaria had been brought under control, but not completely eliminated, in other words, where the possibilities of re-infection are small, new infections develop chiefly among newcomers and relatively infrequently among the cured residents. Celli (1900), and Yorke and Macfie (1924) report instances of natural resistance to experimental malaria infections. There is little room for doubt, therefore, that there exists a type of immunity or resistance to malarial parasites, but the mechanism of this immunity or resistance is still obscure.

An experimental approach to this problem is difficult, because of the specific affinity of the malaria plasmodium for the human host. Consequently we have attempted to study the mechanism of susceptibility and resistance to a protozoan infection, the trypanosome, pathogenic for laboratory animals.

The *Trypanosoma evansi* which we have isolated from infected mules (1924) produces in rabbits a chronic relapsing infection. These animals are all susceptible to the infection and, apart from variations in individual animals, the nature of the disease which develops is fairly constant. There is an initial period of incubation followed by an intermittent fever, accompanied by slow emaciation and culminating in death. Various degrees of host resistance manifest themselves in longer or shorter incubation periods, in a more or less acute course of the disease and in a greater or lesser duration of the illness. Both the parasites and host are well adapted to the study we had in mind.

The Taliaferros (1922) have investigated the question of host resistance by following the course of events in the life-cycle of the invading parasite. They concluded from measurements of the parasites that in the relapsing type of infection the host in some way destroys the parasites, but does not affect the rate of reproduction.

In our study of this problem we have directed our observations particularly to the changes produced in the animal host. In a previous communication (1924) we showed : (1) That by the usual procedure it is not possible to demonstrate humoral parasitocidal antibodies ; (2) that there is a definite change in the leucocyte picture during the course of the disease, (3) that by disturbing the leucocyte balance by the injection of olive oil, a relapse or blood invasion may be produced at will ; and (4) that animals cured with ' Bayer 205 ' acquire an absolute resistance, of greater or lesser duration, to a re-infection.

On the basis of our observations we were led to conclude that the mechanism of infection and resistance, in the rabbit at least, is a resultant of the interaction of two elements, one generated by the host, the other by the parasite, and that the formed elements are chiefly responsible for host resistance. The conclusion reached was summarised by the following statement : ' In the course of the infection the resistance offered by the invasion of the blood stream is repeatedly broken down (probably by substances liberated by the parasites which continue their activity in the tissues), and trypanosomes penetrate into the circulation and go through a period of active development. The destruction of the trypanosomes in the circula-

tion leads to a partial immunization resulting in a disappearance of the parasites from the circulation.'

This assumption pre-supposes a double property of the parasitic cell; one sensitizing, the other immunizing. In this paper we present experimental evidence bearing on this point. The object of these experiments was to ascertain whether by the injection of dead and attenuated parasites it is possible: (1) To simulate the changes observed in the host during the natural course of an infection; and (2) to increase the resistance of the host to infection.

### EXPERIMENTAL.

(A) Sensitization of animals by the injection of dead trypanosomes and trypanosome extracts.

Before presenting the experimental data a word of explanation is required as to the criteria which we have adopted for measuring increased resistance or susceptibility. The disease produced in experimental animals is, as already stated, invariably fatal. Increased susceptibility cannot, therefore, be measured by the percentage of animals dying and recovering from the infection, although that criterion may serve as a measure of increased resistance. In our preliminary experiments we found that the incubation period, that is, the interval between infection and the appearance of the trypanosomes in the circulation, is a dependable measure of the relative degree of that resistance. In other words, we used the resistance offered by the animal to blood invasion as an index of host resistance.

The incubation period as defined above is fairly constant for the rabbit, the animal used in these experiments. Sometimes a careful search may reveal a rare parasite in the peripheral blood within twenty-four hours after the infection. These disappear, however, the next day, and are not found again until the disease has established itself, as shown by the leucopenia and the increase of the mononuclears to about 60 per cent. of the total.

In the blood examinations we always used the thick drop, stained with Giemsa solution (1 : 20) for thirty minutes. At least three drops are examined and the results are recorded as the average number of trypanosomes per microscopic field. This method enables us to gauge roughly the relative intensity of infection as well as the presence

or absence of trypanosomes. The mere presence of a rare trypanosome (less than one in twenty fields) is not considered as a positive blood invasion.

**EXPERIMENT 1.** Heavily infected guinea-pigs were bled into citrate solution, the red blood cells sedimented by centrifugalization at slow speed (600 revolutions per minute, for ten minutes) and the supernatant fluid removed and centrifuged at high speed (1,500 to 2,000) for fifteen minutes. The clear fluid was decanted and the sediment washed with saline, and again centrifuged. The clear supernatant serum and the saline washings were mixed and injected intravenously into rabbits. The sediment was suspended in 20 c.c. of sterile distilled water, frozen and thawed several times and then injected intravenously into rabbits. Each animal received three injections at five-day intervals, and two weeks after the last injection the animals were inoculated with infected guinea-pig blood. The details of the procedure and results are summarised in the protocols given below.

Rabbit	Weight	Material injected	Dates	Date of infection	Incubation	External symptoms	Character of infection
30	grams 1,910	Supernatant fluid	20th July 25th July 30th July	13th Aug.	4 days	Swelling of ears and eyelids 5 to 7 days after infection; marked after 17 days; swelling intermittent.	Moderate intermittent; after first attack blood invasion uncommon.
31	1,720	Supernatant fluid	20th July 25th July 30th July	13th Aug.	3 days	Swelling of ears and eyelids 5 to 7 days after infection; marked after 17 days; swelling intermittent	After first attack of 2 days, blood invasions absent 2 weeks
32	1,350	Autolized sediment	20th July 25th July 30th July	13th Aug.	4 days	Swelling of ears and eyelids; marked after 2 weeks	Severe infection first 5 days; followed by rare, mild recurrences
33	1,600	—	"	"	"	"	"
34	1,760	Control : Supernatant serum; normal g.-p. blood	20th July 25th July 30th July	13th Aug.	8 days	No swelling	Mild intermittent infection at first, later more severe
35	1,675	Control : Sediment; normal g.-p. blood	20th July 25th July 30th July	13th Aug.	10 days	No swelling	Mild intermittent infection at first, later more severe

This experiment was repeated with essentially the same results, irrespective of whether infected guinea-pig or rabbit blood was used, in infecting the treated animals. The rabbits receiving the autolized sedimented trypanosomes or the washings had a shorter incubation

period, a severe blood invasion during the first few days, followed by rare recurrences; while the control animals developed the usual intermittent infection, mild at first and more severe as the disease progressed, after an incubation period two to three times as long as that in the treated animals. In the treated rabbits the swelling of the ears and eyelids, which was sometimes extremely severe, was a constant accompaniment.

**EXPERIMENT 2a.** A second series of experiments was undertaken to ascertain the effect of the repeated injection of whole trypanosomes in increasing the resistance or susceptibility of the host to infection. The material was obtained as before from the blood of heavily infected guinea-pigs. The blood was collected in citrate solution and allowed to sediment overnight. The trypanosomes in the supernatant fluid were sedimented, re-suspended in saline and killed by heating to 54° C. for thirty minutes. The animals were given five sets of daily injections, in five-day periods, with intervals of two days, or a total of 25 injections.

The results are shown in the protocols below. The effect of these repeated injections was even more severe than that produced by the three injections of autolized trypanosomes. The incubation period was greatly reduced, the blood infection during the first few days was severe and swelling appeared promptly with the onset of the illness and then subsided. In general the indication is that the injection of dead or autolized trypanosomes leads to an explosive onset which subsides after a few days, and is then followed by the usual or an even milder course of the disease.

Rabbit	Weight*	Injections	Infected	Incubation	Swelling	Character of infection
50	grams 1,525	25: from 6th Nov. to 9th Dec.	21st Dec. (0.2 c.c. g.-p. blood, 2 tryps. per field)	1 day	Pronounced first day, then subsided; in- termittent; severe few days before death	Severe, continuous 7 days, then only 2 recurrences in a month. Died 40 days after infection
51	1,370	Control	21st Dec. (0.2 c.c. g.-p. blood, 2 tryps. per field)	5 days	Swelling of ears and eyelids moderate after 45 days	Moderate, intermittent. Died after 60 days
52	1,160	5th Dec. to 7th Jan.	21st Dec. (0.2 c.c. g.-p. blood, 2 tryps. per field)	1 day	Pronounced first day; eyelids, ears and mouth; subsided after 5 days	Severe 4 days; then no relapses until 2 days before death, on 3rd Feb.
53	1,075	Serum and washing from material used for g.-p. 52; 5th Dec. to 7th Jan.	—	2 days	Swelling of ears and eyelids on second day; subsided fifth day	Heavy 3 days; then moderate. Died 5th March
54	1,100	Control	—	6 days	None	Heavy, died 2nd Feb.

\* In so far as possible the weights of the animals in any experiment were approximately the same, the variation being 100-150 grams.

EXPERIMENT 2b. This experiment was the same as the previous one, except that larger rabbits were used, and only 20 injections were given; in addition to the normal control there was also a control rabbit, which received injections of sediment from normal guinea-pig blood. The results were essentially the same as those of the previous experiments.

Rabbit	Weight*	Injections	Infected	Incubation	Swelling	Character of infection
90	grams 1,850	Sedimented; heat killed tryps.; 20 injections ended 10th April	22nd April	4 days	Pronounced after 5 days; intermittent	Continuous 6 days, then intermittent
91	2,000	Sediment normal; g.-p. plasma, 20 injections	22nd April	7 days	Slight after 20 days	Moderate intermittent
92	1,900	Untreated	22nd April	8 days	Slight after 16 days	Moderate intermittent

\* In so far as possible the weights of the animals in any experiment were approximately the same, the variation being 100-150 grams.

EXPERIMENT 3. This series of experiments varied from the previous one in the preparation of the injecting material. In order to eliminate the possibility of autolysis which might occur while standing overnight, the citrated blood was promptly sedimented, the trypanosomes in the supernatant fluid thrown down at high speed, re-suspended in saline heated at 54° C. for thirty minutes and injected. The whole operation required about one hour. The number of injections was the same as in Experiment 2a, and the effect essentially the same.

Rabbit	Weight	Number of injections	Incubation	Swelling	Infection
114	grams 1,800	25	2 days	Moderate for 3 to 5 days after infection; then subsided	Moderate intermittent
115	1,700	25	2 days	Moderate for 3 to 5 days after infection; then subsided	Moderate intermittent
116	1,790	None	6 days	None	Moderate intermittent

EXPERIMENT 3b. The same experiment was repeated giving only 10 injections instead of 25. The difference between the control and treated animals was imperceptible.

It seems clear from the results of these experiments that the repeated injection of whole or autolized trypanosomes fails to increase the resistance of the animal, but on the contrary renders it hyper-sensitive in so far as invasion of the peripheral blood stream is concerned. When autolized trypanosomes (obtained by freezing

and thawing) are employed, three injections of moderately large doses suffice to produce a marked hypersensitiveness. The same results may also be obtained by a sufficient number of injections of the supernatant serum and washing of large numbers of whole trypanosomes. But the most striking effect is produced by a long series of injections of whole heat-killed trypanosomes. These injections seem in no way to have modified the later course of the disease, except that the treated animals more often showed marked swelling and oedema, a condition which is found in untreated animals only after the disease has progressed for some time and become chronic.

In order to get some clue to the concomitant changes occurring in the animal host during the course of the infection, we studied the blood changes during the preparatory treatment, pre-infection and post-infection stages, as well as the cellular reaction in the peritoneal cavity subsequent to the infection. The results fluctuated considerably and gave no indication of any significant changes in the white blood counts.

The immediate effect of the injection of trypanosomes, serum, or autolized trypanosomes, was an increase in the total leucocyte count and in the polynuclears. The total leucocyte count was sometimes double the normal. This first reaction was followed by a progressive decrease in the polynuclears and by a proportional increase in the lymphocytes, the total count remaining the same. Each subsequent injection produced the same effect—an immediate rise in the polynuclears followed by a fall. Usually, on about the fifth day after the injection there is a tendency to return to the normal ratio, which is reached in about eight days. Control animals injected with normal guinea-pig serum or sediment show the same general tendency but not to such a marked degree. This disturbance of the white cell ratio is evidently due to the injection of foreign material and is not a specific reaction to the trypanosome cell. The only constant change due to the trypanosome infection is the one previously noted, namely the lymphocytosis, which accompanies the development of the infection, and is most marked during periods of blood invasion.

The changes in the peritoneal fluid were also apparently non-specific. After the first twenty-four hours polynuclears predominate



(70 to 80 per cent.), then there is a progressive increase in the proportion of lymphocytes, so that on the fourth or fifth day they constitute about 90 per cent. of the total. There is no apparent relation between this reaction, the previous treatment of the animal and the activity of the injected trypanosomes in the peritoneal cavity. In some animals the trypanosomes continue multiplying in the peritoneum for several days; in others they disappear from the cavity in twenty-four hours.

(B) Resistance of animals treated with trypanosomes suspended in a solution of 'Bayer 205.'

Failing to induce any sort of immunity by repeated injections of trypanosomes, either intact or autolized, we turned our attention in another direction. Experiments already reported (1924) showed that infected animals cured with 'Bayer 205' developed an immunity to reinfection which was more constant and of longer duration than the resistance of healthy animals treated with the same dose of the drug. It appeared, therefore, that the resulting immunity was induced by a combined action of the trypanosomes and the drug. Consequently a series of experiments was carried out to determine whether a similar effect could be obtained by treating animals with a mixture of dead or attenuated trypanosomes and 'Bayer 205.'

EXPERIMENT 4. Blood from heavily infected guinea-pigs was withdrawn into citrate solution and sedimented, first at low speed to remove the red cells, then at high speed to collect the trypanosomes. The sedimented trypanosomes were re-suspended in saline, and frozen and thawed four times. The material was divided into two parts; one part was injected directly into a rabbit, the second portion was added to a solution of Germanin ('Bayer 205'), kept for half-an-hour at room temperature, and then injected into another rabbit. A control rabbit received an equivalent amount of the drug without trypanosomes, repeated on three consecutive days. The total quantity of drug injected was 0.005 gm. per kilo, an amount shown by our previous experiments (1924) to be insufficient to protect a rabbit against infection. Two weeks after the last injection the animals were infected with the same dose (0.1 c.c.) of guinea-pig blood.

The results were striking. The animal which received injections of trypanosomes alone had a severe explosive infection, similar to those recorded above, resulting in death after fifteen days. Trypanosomes were constantly present in the blood, beginning with the second day.

The rabbit which received only 'Bayer 205' had a prolonged incubation period (ten days), and a moderate infection ending in death on the sixteenth day.

The rabbit which received trypanosomes plus 'Bayer 205' seemed at first, from the nature of the differential leucocyte count, to be developing an infection (on 24th October the differential white cell count was polynuclears 40, mononuclears 60); but it recovered completely, as was indicated by the increased weight of the animal and the return of the normal differential count.

The resistance was of relatively short duration. On 28th November, after the rabbit had completely recovered, it was re-infected and after a somewhat prolonged incubation period of ten days it developed a typical infection, which ran the usual course.

The details of the experiment are tabulated on p. 156.

**EXPERIMENT 2.** The second experiment was similar to the previous one, except that the trypanosomes were not disintegrated by freezing and thawing, but were rendered non-infective by exposing them for twenty-four hours to the action of dilute solution of 'Bayer 205.' Heavily infected guinea-pigs were bled into citrate solution and the mixture centrifugalized at low speed (600 revolutions per minute) for ten minutes. To the clear plasma, containing large numbers of trypanosomes, 'Bayer 205' was added to make a final dilution of 1:400, and the tubes incubated at 25° C. for twenty-four hours. This treatment leaves the trypanosomes intact, but partially or wholly immobilizes them and renders them non-infective. A control rabbit received only 'Bayer 205' in the same dilution and amount. Four injections were given on alternate days.

This experiment was not as conclusive as the previous one, because by an error the total amount of 'Bayer' was 0.01 gm. per kilo instead of 0.005 gm., and this quantity masked the effect of the trypanosomes. Both animals, the one treated with trypanosomes plus 'Bayer 205' and the one treated with the drug alone recovered from the infection, with the difference that no trypanosomes were ever found in the peripheral blood of the former, while in the latter they were present once, on the seventh day of the infection, and then disappeared.

**EXPERIMENT 3.** In this experiment 10 c.c. of heavily infected blood was drawn from guinea-pigs, the red cells sedimented, the plasma decanted and the trypanosomes thrown down at high speed. The sedimented trypanosomes were re-suspended in saline, heated to 55° C. for five minutes, 0.002 gms. 'Bayer 205' added, and the material injected intravenously into a rabbit. Three injections were given on three successive days. A control rabbit received injections of comparable doses (a total of 0.005 gms. per kilo) of 'Bayer 205' alone. Ten days later the animals were infected.

The results are seen in the following protocol. The control rabbit receiving no treatment developed the usual infection after

Rabbit	Weight	Material injected	Dates	Date of infection	Result	White blood counts ; average for five-day period	Differential counts ; average for five-day period	Results
K	grams 820	Sedimented trypsa, frozen and thawed	25th Sept. to 27th Sept.	12th Oct.	14th Oct., blood positive ; heavy infection ; continuous until death, 27th Oct.	7,500 ; 6,500	15-19 20-24 24 + <i>P</i> <sub>38</sub> , <i>M</i> <sub>62</sub> ; <i>P</i> <sub>37</sub> , <i>M</i> <sub>62</sub> ; <i>P</i> <sub>35</sub> , <i>M</i> <sub>65</sub>	Died 27th Oct.
L	895	Trypsa as above ; + total 0.005 gm. 'Bayer 205' per kilo.	25th Sept. to 27th Sept.	12th Oct.	Infection negative ; animal gained in weight	7,300 ; 7,400 ; 7,400	<i>P</i> <sub>48</sub> , <i>M</i> <sub>52</sub> ; <i>P</i> <sub>44</sub> , <i>M</i> <sub>55</sub> ; <i>P</i> <sub>43</sub> , <i>M</i> <sub>57</sub> ; <i>P</i> <sub>40</sub> , <i>M</i> <sub>54</sub> ; <i>P</i> <sub>52</sub> , <i>M</i> <sub>48</sub>	Discharged 20th Nov. ; Weight, 1,085 gm. ; W. B. C. 9,300 ; Differential, <i>P</i> <sub>55</sub> , <i>M</i> <sub>45</sub>
M	850	Only 'Bayer 205' ; total 0.005 gm. to per kilo. given in 3 injections of 0.0014 gm.	25th Sept. to 27th Sept.	12th Oct.	Blood positive, 22nd Oct., after 10 days	7,800 ; 7,800	<i>P</i> <sub>50</sub> , <i>M</i> <sub>50</sub> ; <i>P</i> <sub>43</sub> , <i>M</i> <sub>57</sub>	Died 28th Oct.

*P* = Polynuclears.

*M* = Mononuclears.

an incubation period of seven days, the disease following the regular course with a fatal ending on 20th June (88 days). In the rabbit treated with 'Bayer 205' alone trypanosomes appeared in the peripheral circulation six days after the inoculation and the disease followed the usual course. The animal died on 4th May, from an intercurrent infection.

The rabbit treated with trypanosomes plus 'Bayer' had a prolonged incubation period of fourteen days and a milder infection which ended on 15th July (113 days).

In this experiment there is again an absence of the hypersensitive condition noted in animals treated with trypanosomes alone, and a degree of resistance not observed in the control animal, treated with the corresponding dose of the drug without trypanosomes, despite the fact that the latter was larger and consequently possessed greater natural resistance.

Rabbit	Weight	Treatment, 11th March to 13th March	Infected	Incubation	Death	Nature of infection
56	grams 1,300	None	24th March	7 days	88 days	Moderate
57	1,900*	Three injections of 'Bayer 205' totalling 0.005 gm. per kilo.	24th March	6 days	Intercurrent infection, 56th day	Moderate
58	1,280	Three injections of trypanosomes heated at 55° C., plus 'Bayer 205,' totalling 0.005 gm. per kilo.	24th March	14 days	113 days	Mild

\* An animal of the same weight was not available at the time of the experiment and consequently a larger rabbit was used as the drug control.

**EXPERIMENT 4.** This experiment followed the same general plan as the previous one, with variations in details. Two rabbits received ten daily injections of trypanosome suspensions in saline. The trypanosomes were obtained from infected guinea-pigs, sedimented, suspended in saline and killed or attenuated by incubating at 37° C. for twenty-four hours. Two more rabbits received the same number of injections of material prepared in the same way, except that 'Bayer 205' was added in amounts so graduated that the total quantity injected should not exceed 0.005 gm. per kilo. A fifth rabbit received ten daily injections of 'Bayer 205', totalling 0.005 gm. per kilo.

The results corresponded with those obtained in the previous experiments. The animals treated with trypanosomes alone developed the blood infection on the fourth and fifth day after the

inoculation, with mild swelling of the eyelids. Those receiving both trypanosomes and 'Bayer' developed the blood infection only after nineteen and twenty-one days respectively and swelling appeared only towards the end of the disease. The animal treated with small doses of 'Bayer 205' alone developed the infection on the eleventh day.

Rabbit	Weight	Treatment	Duration of treatment	Infected	Onset of disease*	Death
64	grams 1,170	Ten injections killed trypanosomes	14th April to 25th April	6th May	10th May	29th May, inter-current infection
65	1,000	Ten injections killed trypanosomes	14th April to 25th April	6th May	11th May	12th June, typical
66	1,250	Ten injections trypanosomes plus Bayer	14th April to 25th April	6th May	25th May	3rd July, typical
67	1,050	Ten injections trypanosomes plus Bayer	14th April to 25th April	6th May	27th May	24th June, inter-current infection
68	1,100	Ten injections 'Bayer 205'	14th April to 25th April	6th May	17th May	15th July, typical

\* Trypanosomes in peripheral circulation.

## DISCUSSION

On account of technical difficulties experienced in collecting large amounts of trypanosomes with the primitive apparatus available, these experiments had to be temporarily suspended. Enough work has been done, however, to indicate that trypanosomes suspended in 'Bayer 205' behave quite differently from those suspended in saline and that the animals so treated, instead of becoming hypersusceptible, develop a resistance quite distinct from that induced by the same quantity of the drug alone.

The experiments reported above give a clue to the nature of some of the pathological changes noted in trypanosome infection, at least in so far as they manifest themselves in experimental animals. The mechanism of the disease is evidently a complex one. First there is the unexplained mystery of the appearance and disappearance of parasites in the blood stream, then there are the late

manifestations of the disease, varying from mild swelling and oedema to severe ulceration and necrosis.

In so far as the experiments reported in this paper simulate the changes occurring in the infected animals, it appears that the manifestations of the disease are due to a progressive sensitization of the host by the parasite or its product. The periodic invasion of the blood stream seems to depend on the repeated breaking down of some barrier by the trypanosomes multiplying in the animal organism. Just as the repeated injection of whole, or disintegrated, trypanosomes sensitizes the animals so that when infected the invasion of the circulation is prompt and, in small rabbits, even continuous, so in infected normal animals a period of incubation is required for sensitizing the animal before the peripheral circulation can be invaded.

Similarly the appearance of swelling and oedema is due to a prior sensitization by the trypanosomes or their products. In healthy animals this condition can be produced artificially by the injection of the same strain of trypanosomes, killed, attenuated or disintegrated. In animals previously so treated, swelling of the ears and eyelids occurs promptly after the infection simultaneously with the invasion of the circulation. In normal animals swelling occurs only late in the disease, the fourth or fifth week ; or, in other words, after the animal has become spontaneously sensitized by the absorption of the parasites or their products. The oedema and swelling noted in the later stages of the disease are, therefore, direct effects of the parasite and its products.

If, however, the condition of treatment is varied and the trypanosomes are injected together with the drug 'Bayer 205,' an exactly opposite state is established in the animals. Instead of being hypersensitive, the animals manifest an increased resistance which in some of them may suffice to ward off an infection. This acquired resistance cannot be attributed to the protective action of the drug, because the same dose of the drug without trypanosomes has little or only a mild protective action.

The same phenomenon has already been observed (1924) in infected animals treated with 'Bayer 205.' Animals cured with this drug manifest a degree of resistance to re-infection greater and of much longer duration than normal controls treated with corresponding doses of the drug.

It is difficult to imagine what peculiar transformation is brought about by the drug which results in such markedly different reactions. It would appear that the drug combines in some way with the trypanosome cell, producing a heterogenic antigen capable of stimulating the formation of specific antibodies.

This may possibly be a demonstration of the type of a combined chemico-immuno-therapy supposed by Yorke (1925) to be going on in malaria patients treated with quinine. The latter drug is just as specific for the malaria plasmodia as 'Bayer 205' is for trypanosomes. The action of both these drugs is not, however, purely germicidal; associated with this property there is apparently also an immunizing activity which completes the cure and affords an increased resistance of greater or lesser duration to subsequent infection. Whether or not artificial immunization would result from the treatment of suitable animals with *Haemosporidia* and quinine remains to be determined; the experiments reported here, incomplete though they admittedly are, suggest that animals may be rendered more resistant to trypanosome infection if treated with a mixture of trypanosomes and 'Bayer 205.'

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# A SECOND CASE OF SLEEPING SICKNESS IN THE SUDAN CAUSED BY *TRYPANOSOMA RHODESIENSE*

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Early this year, some films representing gland juice smears, taken from a case of sleeping sickness in the Tembura district of the Bahr el Ghazal, were sent to these laboratories for examination. Accompanying the smears were also blood films taken from white rats which had been inoculated with the gland fluid obtained by gland puncture of a cervical gland from the same case of sleeping sickness.

The gland juice smears were fixed and stained, and a fair number of trypanosomes found. The blood smears from the rats were fixed and stained and examination showed the presence of trypanosomes, some of which showed posterior nucleated forms indistinguishable from *T. rhodesiense*.

The history of the case was sent by Capt. Maurice, R.A.M.C., Senior Medical Officer of Sleeping Sickness in the Southern Bahr el Ghazal. The patient, No. 3324 Combanuga, was a boy aged fifteen, belonging to the Sueh region further north than half-way between Tembura and the Bo river, a district where *G. morsitans* was plentiful. He was first seen on December 6th, 1924, by Capt. Maurice, who stated that the patient had the facial appearance of a case of sleeping sickness in its acute form but that his general condition was good. The cervical glands were small, and shotty in consistence; gland puncture confirmed the diagnosis of sleeping sickness. On the same date, 0.5 grm. and a week later, 1 grm. of atoxyl was given intramuscularly. Ten days later, the gland juice was again examined and still larger numbers of trypanosomes were found than at the previous examination. As this was somewhat



unusual in the experience of Capt. Maurice, trypanosomes usually disappearing from the gland juice for a period of a month after 1.5 grm. atoxyl, smear preparations of the gland juice were prepared and a rat inoculated, and blood films from the rat sent to these laboratories for examination. The result of this examination has already been referred to. Its importance was realised and Capt. Maurice was asked to inoculate further rats with the gland juice and blood of the case and despatch them in fly-proof cages to Khartoum. The inoculations were carried out and the rats brought to Khartoum in the charge of a British medical officer. In the meantime, the patient, who had been under treatment, had rapidly lost ground, and, at the time of the inoculations, was in the final stage of the disease and moribund, and indeed died shortly afterwards, within a period of six months after being diagnosed and treated.

The rats reached these laboratories twenty-nine days after they had been inoculated. One had been inoculated with 3 c.c. of centrifuged blood obtained from the patient and the other had been inoculated with the gland fluid obtained by puncture of the cervical glands. Both rats arrived infected with trypanosomes, some of which showed posterior nucleated forms.

Sub-inoculations were carried out and the strain from the rats maintained in rats and other animals for further investigation.

In the meantime, the original gland juice smears and blood films from the rat inoculated by Capt. Maurice in the Tembura district were submitted to Dr. Wenyon for examination together with notes of the sleeping sickness case. Dr. Wenyon reported as follows: 'The films from the rat show what I regard as typical *Trypanosoma rhodesiense* with a high percentage of posterior nuclear forms. Certainly the history of the case seems to support this view. I think there can be no doubt that this type of Trypanosomiasis must occur in the Southern Sudan.'

The rat inoculated with 3 c.c. of centrifuged blood from the patient survived for a period of 31 days, while the rat inoculated with the gland juice died 42 days after inoculation. The peripheral blood of both animals showed an intense infection with trypanosomes, long and slender forms as well as short and broad forms; the latter were present in large numbers averaging about 50 per cent. of all the trypanosomes, and about 20 per cent. of the short forms showed

various stages of posterior displacement of the nucleus. Two per cent. showed the nucleus at the extreme posterior extremity of the trypanosome. In the smear preparations of the gland juice of the patient no posterior nucleated trypanosomes were found.

Three peripheral blood films taken from the patient when in a moribund condition were also examined, but no trypanosomes were found.

### PATHOGENICITY

Sub-inoculations from the two infected white rats were carried out in the small desert rat (*Gerbillus gerbillus*) and the strain maintained for further investigations, as already stated.

The original white rat inoculated with the gland fluid from the cervical glands of the patient survived 42 days, and the white rat inoculated with the centrifuged blood of the patient survived 31 days.

Sub-inoculations in rats and other animals gave the following results :—

Animal	Incubation	Duration of life
White rat ... ..	5-6 days	30-35 days
<i>Gerbillus gerbillus</i> ... ..	4-5 days	10-44 days
Rabbit ... ..	7-8 days	42 days
Monkey ( <i>C. sebaeus</i> ) ... ..	5 days	17 days
Guinea-pig ... ..	18 days	53 days
Lizard ( <i>Varanus niloticus</i> ) ... ..	—	Still alive and uninfected after 60 days

Sub-inoculations in white rats and gerbils showed the presence of posterior nucleated forms. Examination of these showed that their percentage was less than in the first inoculated rat, constituting from 5 to 13 per cent. of the stout forms of trypanosomes.

Serological and immunity tests were not performed as the laboratory strain of *T. gambiense* had died.

## CULTIVATION ON ARTIFICIAL MEDIA

Citrated blood from infected rats was inoculated into the water of condensation of cultures of Novy-MacNeal-Nicolle medium and incubated at 22° C. to 24° C. Cultures examined on the fourth day showed free, actively motile, thin forms of trypanosomes. On the eighth day these had apparently multiplied, aggregations of dividing forms being found in fresh preparations.

Sub-cultures were successfully maintained for two generations, aerial contamination eventually killing the cultures.

White rats and gerbils inoculated with cultures five days old failed to become infected.

## REMARKS

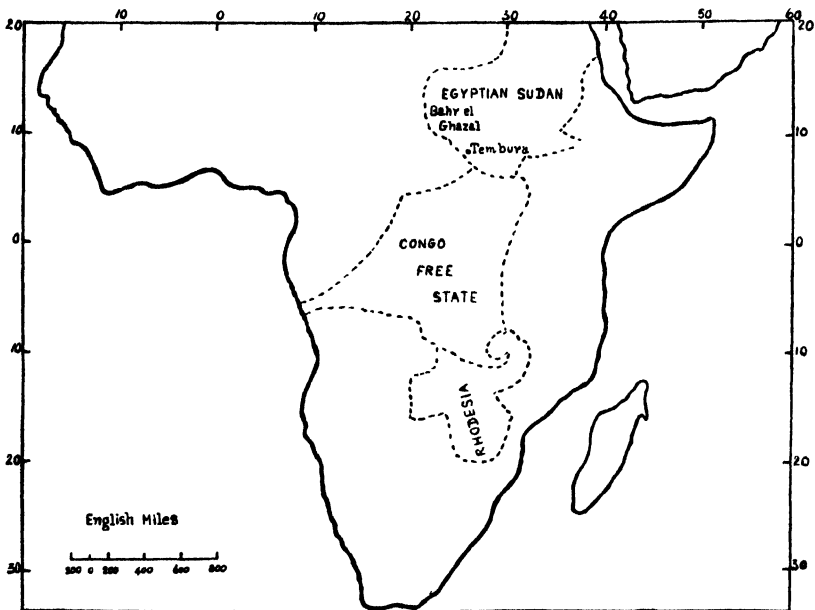
The clinical history of this case of human trypanosomiasis, and the morphological characters of the trypanosome in the blood of rats inoculated with the gland juice of the patient, support the view that this was a case of human trypanosomiasis caused by *T. rhodesiense*, a hypothesis which received further confirmation by testing the pathogenicity of the trypanosome in the white rat, gerbil, monkey, rabbit, and guinea-pig. As already mentioned, the posterior nucleated forms were not found in the gland juice of the patient but appeared in an unusually high percentage in the blood of inoculated rats, constituting nearly 20 per cent. of the short broad forms of the trypanosomes in the first series of inoculated rats. Sub-inoculations carried out in these animals showed that the posterior nucleated forms averaged from 5 per cent. to 13 per cent. of the short broad forms, the percentage varying with the duration of the infection in the inoculated rats.

The previous record of *T. rhodesiense* in the Sudan (1922) was a case of human trypanosomiasis in a native of the same district of the Bahr el Ghazal; gerbils inoculated with the gland juice from this case showed the presence of posterior nucleated forms in peripheral blood films, these forms occurring also in sub-inoculated white rats and a dog. The pathogenicity of this trypanosome was tested in animals by Major Whitehead, M.C., R.A.M.C., and the following results obtained :—

Animal	Incubation	Duration of life
Gerbil ... ..	5-7 days	18-23 days
White rat ... ..	4 days	14 days
Rabbit ... ..	5 days	15 days
Guinea-pig ... ..	12 days	34 days
Monkey ... ..	5 days	18 days
Dog ... ..	4 days	6 days
Goat ... ..	10 days	Alive after 28 days

The measurements of the trypanosome were very similar to those of the second case, varying from 10 to 30  $\mu$ .

The pathogenicity of the trypanosome from the two cases certainly resembles that of *T. rhodesiense*.



The two cases are of interest inasmuch as they occurred in natives of the Tembura district of the Bahr el Ghazal, a sleeping

sickness area in which *T. gambiense* is endemic and in which the tsetse flies *G. fuscipes* and *G. morsitans* abound.

Thanks to the energetic and, it may be added, successful measures for dealing with sleeping sickness, the malady in the Bahr el Ghazal appears to be under control, but the danger of infected cases crossing the western frontier from adjacent territories is a real one, necessitating close co-operative measures with the countries concerned. So far, the common form of human trypanosomiasis has been caused by *T. gambiense*, but the authorities are fully alive to the possibilities of *T. rhodesiense* occurring in man in a country where *G. morsitans* is almost ubiquitous and where game and stock act as reservoirs of *T. pecaui vel brucei*. The two cases reported indicate so far that human trypanosomiasis caused by *T. rhodesiense* exists in the Bahr el Ghazal, happily only in sporadic form.

### CONCLUSIONS

1. A short account is given of a second case of human trypanosomiasis caused by *T. rhodesiense* occurring in a native of the Bahr el Ghazal district of the Sudan.
2. The clinical history of the case resembled that of *T. rhodesiense* in man.
3. Rats inoculated with the gland juice of the patient showed a high percentage of posterior nucleated forms of trypanosomes.
4. The pathogenicity of the trypanosome for laboratory animals closely resembles that of *T. rhodesiense*.

### REFERENCE

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# OBSERVATIONS ON THE DEVELOPMENT OF HOOKWORM LARVAE

## PART II

BY

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*(Received for publication 16 February, 1926)*

The technique employed in the following experiments is exactly the same as that described in Part I of this paper, and the results of the experiments are all expressed as the number of larvae isolated per gram of faeces employed. It will also be noted that in the various series of experiments the numbers of larvae recovered are very different; this is because faeces from different individuals were used.

### EXPERIMENT 4.

#### *The effect of urine on mixtures of soil and faeces.*

In this experiment three grams of faeces were mixed with laterite in the ordinary way, being kept moist by the daily addition, for a period of seven days, of sufficient fresh urine for the purpose. Control cultures had equal quantities of water added.

#### SERIES 1.

Urine-moistened cultures produced	...	...	...	...	6 larvae.
Control cultures produced	...	...	...	...	1,736 larvae.

#### SERIES 2.

Urine-moistened cultures produced	...	...	...	...	22 larvae.
Control cultures produced	...	...	...	...	144 larvae.

The larvae isolated from the urine-moistened cultures were very small and were degenerate in appearance, and it is doubtful if any of them were infective. They were placed in tap water to test their viability, and all were dead within forty-eight hours, whereas larvae from the controls showed no diminution in numbers living or in activity, at the end of the same period.

## EXPERIMENT 5.

*The effect of the immersion of faeces in different liquids for varying periods.*

In this series of experiments the faeces were immersed in the different liquids for the times stated; they were then mixed with laterite and cultured for seven days in the routine manner.

## EXPERIMENT 5a.

*Effect of prolonged immersion in tap water without disintegration of faeces.*

Portions of faeces, each of about ten grams in weight, were tied in small pieces of muslin and each packet was placed in a separate beaker of tap water. The packets of faeces floated partly submerged in the water. At the end of the periods stated in the table below, the water in which the faeces were immersed was examined for the presence of larvae, three grams of faeces were taken and mixed with laterite for culture, and the balance of the faeces was placed direct into the isolation apparatus to see if larvae were present.

No.	Time immersion was continued	Larvae in water of immersion	Eggs in faeces	Larvae in faeces	Yield of larvae from culture
1	1 week	o	+	o	37
2	2 weeks	o	+	o	44
3	3 weeks	o	+	o	53
4	4 weeks	o	+	o	35
5	5 weeks	o	+	o	39

Even after five weeks' immersion the faeces looked just as fresh as when first placed in the muslin and the eggs showed no apparent development.

## EXPERIMENT 5b.

*The effect of the immersion of faeces in different liquids for varying periods.*

Equal portions of faeces from a well-mixed stool were placed in beakers; they were not tied in muslin, so the various liquids added

could act freely on them. The liquids used were tap water, a disinfectant containing 1 per cent. free chlorine, sea water, and fresh urine. The faeces became saturated and sank to the bottom of the beakers in about twenty-four hours. After varying periods, indicated in the table below, the liquids were drawn off with a pipette as completely as possible. None of the liquids contained any larvae. Portions of the faeces, three grams in weight, were now mixed with laterite and were cultured for seven days. During the period of growth they were all kept moist with tap water, irrespective of the liquid in which they were originally immersed; by keeping the cultures under identical conditions in this manner, the lethal effect of the liquids on the eggs and not on the young larvae could be determined.

Liquid in which immersed	Number of days immersion lasted				
	1	2	4	7	9
Tap water ... ..	4,337	Culture lost	3,350	2,460	2,837
Sea water ... ..	4,730	Culture lost	4,223	1,393	1,287
Disinfectant ... ..	5,640	2,967	2,460	2,260	2,857
Urine ... ..	4,680	4,607	3,983	2,837	2,360
	Larvae isolated per gram of faeces				

Control cultures put up on the day on which immersion was commenced produced 6,097 larvae. The greater number of larvae in the controls is probably due not to the death of some eggs after only one day's immersion, but rather to the unavoidable dilution of the faeces after immersion.

#### EXPERIMENT 5C.

##### *Effect of immersion for prolonged periods in various liquids.*

Except that the immersion of the faeces in the liquids was continued for longer periods this experiment is identical with Experiment 5b. Moulds growing on the surfaces of the liquids



were removed with a section-lifter, care being taken not to disturb the faeces lying at the bottom of the beakers.

Fluid in which immersed	Number of days immersion lasted					
	7	14	18	21	28	35
Tap water ... ..	177	127	150	138	27	0
Sea water ... ..	127	100	80	27	0	0
Urine ... ..	140	0	0	0	0	0
Larvae isolated per gram of faeces						

Cultures made from the same specimen of faeces on the day on which immersion was commenced produced 583 larvae. As in Experiment 5b, the greater number of larvae recovered from the controls is to some extent probably due to dilution of the faeces, but the difference between the controls and immersed faeces is more marked in this instance because the first examination was not made until after a week's immersion, at which time, judging by analogy with Experiment 5b, about half the eggs would be dead even in tap water.

#### EXPERIMENT 5d.

##### *Effect of mixing faeces with soil before immersion.*

Portions of faeces, three grams in weight, were mixed with 25 c.c. of laterite or sea sand, placed in beakers, and covered with either tap water, sea water or urine. The beakers were allowed to stand for fourteen days; this time was chosen on account of the results obtained in Experiment 5c. At the end of this time the liquids were pipetted off and examined for the presence of larvae, but none were found in any instance. The whole of the soil and faeces mixtures was now transferred to wire baskets and they were at once tested by hot water isolation. The baskets were then allowed to drain off any excess of water and were kept for a further seven days, being kept moist with tap water meanwhile. At the end of this time they were again subjected to isolation with hot water. The results are given in the table below.

	Liquid in which immersed		
	Tap water	Sea water	Urine
First isolation— Laterite mixture ... ..	0	0	0
Sea sand mixture ... ..	0	0	0
Second isolation— Laterite mixture ... ..	80	253	0
Sea sand mixture ... ..	127	201	0
Larvae isolated per gram of faeces			

Control cultures made on the day on which the experiment was commenced produced 820 larvae per gram of faeces. In this instance dilution of the faeces does not partially explain the difference, for the whole of the contents of each beaker was subjected to isolation and it was noted that no eggs were lost from the culture during the first isolation.

#### EXPERIMENT 5e.

##### *Effect of saturation in different liquids without immersion in them.*

This experiment is the same as Experiment 3b in Part I of this paper, except that urine was used in addition to tap water and sea water. For the sake of convenient comparison the figures in Experiment 3b are here reproduced, together with those obtained from the use of urine under the same conditions.

Series 1 (kept for 7 days).

	Liquid in which saturated		
	Tap water	Sea water	Urine
First isolation— Laterite ... ..	50	53	53
Sea sand ... ..	7	17	0
Second isolation— Laterite ... ..	67	7	7
Sea sand ... ..	197	153	0

Series 2 (kept for 14 days).

	Liquid in which saturated		
	Tap water	Sea water	Urine
First isolation—			
Laterite ... ..	230	137	0
Sea sand ... ..	20	7	0
Second isolation—			
Laterite ... ..	0	0	0
Sea sand ... ..	203	17	0
	Larvae isolated per gram of faeces		

Control cultures of the same faeces put up on the day on which the experiment was commenced produced 820 larvae per gram of faeces.

### DISCUSSION

This series of experiments shows that urine has a definite effect in destroying both hookworm eggs and freshly-hatched larvae. In Experiments 5b and 5c it will be noted that it is somewhere between nine and fourteen days before all the hookworm eggs in faeces are killed by urine. In Experiment 4 the eggs must have been in a condition to hatch out, so it must be assumed that urine-moistened soil will kill young larvae. Minagawa (1919), working with *Ancylostomum caninum*, showed indirectly that urine killed the larvae of this species, for he failed to infect young dogs that were placed on soil that had been heavily infected with faeces containing eggs of *A. caninum*, and which had been moistened with urine previously, whereas in his control experiments the dogs became infected. The inference drawn by Minagawa was that the larvae of *A. caninum* were killed by contact with human urine, and the writer's experiment shows that the same applies to the larvae of hookworms infecting human beings.

In Experiment 5a, it will be seen that the packets of faeces were only partly submerged in water, and the result was that after five weeks there was no death of hookworm eggs; in Experiment 5c, however, in which the faeces were totally excluded from contact with air, all the eggs failed to hatch after the same period of immersion.

Khalil (1922) found apparently normal undeveloped hookworm eggs in the effluent of septic tanks in British Guiana, and he concludes from this that septic tanks of the type investigated are of no use in preventing the spread of Ankylostomiasis except by concentrating the infective material into one channel. As there was apparently no way of knowing how many eggs entered the tanks, it is not possible to say if any were killed during their passage through the system. Experiment 5b in the present series shows that after four days' complete immersion in water about 25 per cent. of the eggs were destroyed, and Experiment 5c shows that there is a steady diminution in the number of viable eggs recoverable from water as immersion is continued, until after a period of five weeks all the eggs seem to be dead. From these observations it seems safe to conclude that septic tanks would have some effect in reducing hookworm infection, the actual reduction being directly dependent on the time the faeces remained in the tank.

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# FURTHER OBSERVATIONS ON THE TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN FROM *PHLEBOTOMUS PAPATASII*

BY

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## PLATES XVI-XVIII

From October to December, 1924, and from April to December, 1925, sandflies were systematically collected in Jericho, with the object of determining the infection rate of sandflies with *Herpetomonas* in an endemic centre of cutaneous Leishmaniasis.

In 1924 three female *P. papatasii* were found positive (out of a total of two hundred and twenty, of which one hundred and seventy-four were females) and it was expected that systematic collection and dissection of a large number of sandflies during 1925 would yield ample material for a study of *Herpetomonas papatasii*.

During 1925, three thousand eight hundred and fifty sandflies from Jericho were dissected. Of these

132 were *P. papatasii* ♂♂

3,624 were *P. papatasii* ♀♀

13 were minutus group ♂♂

81 were minutus group ♀♀.

Three distinct species are included in the minutus group, *P. minutus*, *P. africanus* (syn. *P. minutus* var. *africanus* Newstead, 1912) and one undetermined species. A number of males of *P. papatasii* and of the minutus group were mounted and are not included in the above figures.

Of the total number of sandflies from Jericho only four (*P. papatasii* ♀♀) were found to contain *Herpetomonas*, i.e., an infection rate of, roughly, one per thousand. The result is rather

disappointing, particularly as Wenyon (1912), working in Aleppo, found 6 per cent. of sandflies infected with *Herpetomonas*. (During 1925 only four cases of cutaneous Leishmaniasis, diagnosed first clinically and then microscopically, were found in Jericho.)

The sandflies containing *Herpetomonas* were all captured in houses where there were no clinical cases of cutaneous Leishmaniasis, but it cannot be inferred from this (in spite of the fact that sandflies seldom if ever pass from house to house), that the source of infection in the sandflies was non-human, for, as we shall show later, an insignificant papule, clinically not recognisable as cutaneous Leishmaniasis, may nevertheless contain Leishman-Donovan bodies. Such papules would be overlooked, particularly in localities where insect bites are common.

In addition to the sandflies from Jericho, two hundred and fifty sandflies from Jerusalem, one hundred and eighty from Mozza, and one hundred and twenty from Haifa (all *P. papatasi* females) were dissected, and all were found negative for *Herpetomonas*.

The sandflies were collected in Jericho by one individual and about five hours were spent on each occasion in collecting from as many houses as possible, so that each catch was fairly representative of the *Phlebotomus* population of Jericho except that, as far as possible, females were caught. On April 20th, 1925, one hundred and seven sandflies were captured, and from April 30th to November 17th, 1925, an average catch amounted to about two hundred sandflies, the number varying from one hundred and seventy to two hundred and forty-six. Between November 17th and November 27th, sandflies diminished in numbers rapidly in Jericho, for on the former date two hundred and nine were captured, and on the latter date only one hundred and nine were caught. On the 7th December, 1925, only twenty-two were caught, and these were not found free in rooms, but were poked out of cracks in walls.

#### THE MORPHOLOGY OF *HERPETOMONAS* IN NATURALLY INFECTED SANDFLIES

Of the total of seven positive sandflies found in Jericho during 1924 and 1925, four contained mammalian blood and three contained no blood at all. Material from each of the latter was used for inoculation experiments on man.

The morphology of the *Herpetomonas* was studied in the fresh and in alcohol-fixed preparations stained with Giemsa.

The parasites show an extraordinary degree of polymorphism which renders their classification difficult. They may be divided into two distinct groups, one being a-flagellar and the other possessing a flagellum.

#### FORMS WITHOUT A FLAGELLUM

1. Leishmaniform bodies with one or two nuclei and one or two blepharoplasts. These bodies were found both in the stomach and, in one instance, in a smear of the upper part of the cardia (Pl. XVI, figs. 1-11).

2. Leishmaniform bodies,  $3\mu$  by  $4\mu$ , containing a single nucleus, a blepharoplast and a rhizoplast. These bodies were found in one instance in a smear of the upper part of the cardia containing the oesophageal valve (Pl. XVI, figs. 12 and 13).

3. Large round or oval parasites,  $5\mu$  to  $12\mu$  by  $5\mu$  to  $8.5\mu$  (Pl. XVI, figs. 14-25), with a varying number of nuclei and blepharoplasts. The evolution of this type appears to be as follows; a large round body is found containing a central nucleus and one blepharoplast (Pl. XVI, fig. 14); the blepharoplast divides, and then division of the nucleus follows; in some instances the blepharoplast divides several times before nuclear division commences; finally a protoplasmic mass containing a varying number of nuclei and blepharoplasts is formed; these masses must be considered as schizogony forms, for ultimately the protoplasm divides and a number of merozoites, flagellated and non-flagellated, are produced; in some cases the flagellae of the daughter flagellates appear before division of the protoplasm is complete. Similar forms are found in cultures of *H. tropica* a day after the addition of specific immune serum to the culture.

The above form was found in a smear of the upper end of the cardia.

None of the above a-flagellar forms which were observed possessed a cyst wall; they were all found together in a smear of the upper end of the cardia of a sandfly which was caught on the 26th of October, 1925, and dissected on the 2nd of November, 1925. Between these



two dates the sandfly had no opportunity of feeding. Forms No. 1 were also found in the stomach of sandflies which contained no parasites in the cardia; these facts, taken in conjunction with the results obtained from artificially infected sandflies, lead to the conclusion that form No. 3 is a late stage in the development of the *Herpetomonas* in the sandfly.

### FORMS WITH A FLAGELLUM

The classification of these forms must be artificial, for the various types tend to merge one into the other and there is no sharp dividing line between them. The following types may, however, be distinguished:—

1. Short, very thin forms (body  $6.3\mu$  to  $8.5\mu$  by  $0.4\mu$  to  $0.8\mu$ ), with a flagellum up to  $21\mu$  long (Pl. XVI, figs. 40-45).

2. Long, very thin forms in which the body is scarcely thicker than the flagellum. Body  $12.5\mu$  to  $17\mu$  by  $0.4\mu$  to  $0.8\mu$ . Flagellum up to  $21\mu$  long (Pl. XVI, figs. 46-48).

These forms were observed in the stomach. They move very rapidly across the field, and, owing to the fact that their bodies are flexible and scarcely thicker than the flagellum, they present, on examination in fresh preparations, a superficial resemblance to long, thick spirochaetes.

3. Small, round or pyriform bodies with a short flagellum (body  $3\mu$  to  $3.3\mu$  by  $1.7\mu$  to  $2.5\mu$ ) (Pl. XVI, figs. 26-28). These were found in a smear of the upper part of the cardia.

4. Large, round or spindle-shaped bodies ( $5\mu$  to  $13\mu$  by  $3.3\mu$  to  $8\mu$ ), with a flagellum up to  $24\mu$  in length (Pl. XVI, figs. 29-38). These forms were observed throughout the whole of the midgut.

5. Irregular forms filled with numerous vacuoles and chromatic granules (Pl. XVI, fig. 39). Although these appear to be degenerating forms they are, nevertheless, capable of active division.

6. Long thick flagellates (body  $20\mu$  to  $23\mu$  by  $2.5\mu$  to  $3\mu$ ), with a flagellum up to  $30\mu$  long. These forms are specially numerous in the cardia. They were found in one instance in the proboscis, lying coiled up in chains along the upper surface of one mandible (Pl. XVI, figs. 70 and 71).

7. Intermediate forms (body  $8.5\mu$  to  $15\mu$  by  $1\mu$  to  $3\mu$ ), with a flagellum up to  $18\mu$  (Pl. XVI, figs. 53-64).

The position of the nucleus in the flagellar types is very variable ; usually it is central or almost central, but it may be anterior (Pl. XVI, fig. 46) or posterior (Pl. XVI, fig. 59).

Division of the flagellar types of the *Herpetomonas* from sandflies takes place as follows ; the blepharoplast divides and this division is followed by the formation of a new additional flagellum usually much shorter than the old flagellum ; subsequently the nucleus divides, and then the body divides longitudinally from before, backwards along two fine, closely-set lines which appear to run throughout the whole length of the body.

8. Multiple division forms were found in one sandfly in a smear of the upper part of the cardia. In these forms the blepharoplast and nucleus divide a number of times, the original flagellum is retained or may become rudimentary, and ultimately division of the protoplasm takes place and a number of flagellates are produced (Pl. XVI, figs. 72-75). (Such forms are also found in old cultures on Noguchi medium or its modifications according to Wenyon or Kligler.)

Clusters of flagellates (Pl. XVI, fig. 76) were found in the cardia. Numbers of all types of *Herpetomonas*, flagellated and non-flagellated, were found to contain vacuoles and chromatic granules in their protoplasm.

#### **DISTRIBUTION OF THE *HERPETOMONAS* IN THE ALIMENTARY TRACT OF *P. PAPATASII***

In four of the positive sandflies (those containing mammalian blood) the majority of the *Herpetomonas* were found mainly in the stomach. In the other three they were found in the oesophagus, oesophageal diverticulum, midgut and hindgut. The majority of the flagellates occurred in the cardia, particularly in the upper part of it ; they were found attached in large numbers by their flagella to the oesophageal valve and the epithelium of the cardia. In one of the sandflies they were also found in the pharynx, buccal cavity and the proboscis.

## EXPERIMENTS ON THE TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN

### EXPERIMENT NO. 1 (previously recorded)

*P. papatasi* ♀, caught in Jericho, 25.6.25. Dissected 26.6.25. The whole alimentary tract behind the pharynx contained flagellates, the majority of which were long and rather thick forms (flagellar forms type 6). They were particularly numerous in the upper part of the cardia, where large numbers were attached to the posterior surface of the oesophageal valve.

Material was inoculated into two scarified points on the left forearm of a volunteer.

*Result.* 31.7.25. A small papule was noted on one inoculated point and on examination was found to contain Leishman-Donovan bodies. Early in December, 1925, the papule commenced to scale. Seven and a half months after the inoculation the papule was 2.0 mm. in diameter. Dr. H. Dostrowsky, dermatologist to the Rothschild Hospital, examined the papule and stated that, clinically, it could not be diagnosed as oriental sore. Nevertheless, Leishman-Donovan bodies were still present (8.2.26), though cultures remained negative.

### EXPERIMENT NO. 2

*P. papatasi* ♀, caught in Jericho, 8.9.25. Dissected 9.9.25. *Herpetomonas* found in oesophagus and oesophageal diverticulum, midgut and hindgut. The parasites were swarming in the cardia, where large numbers were found attached to the epithelium of the cardia and to the oesophageal valve.

*Types of parasites:* Flagellated types 1, 4 and 7.

Material from this sandfly was inoculated into two scarified points on the left forearm of a volunteer. After two weeks all traces of the original inoculation had disappeared. The case was observed till 15th December, 1925, and the inoculated points were found negative for Leishman-Donovan bodies.

### EXPERIMENT NO. 3

*P. papatasi* ♀, caught 26.10.25, dissected 2.11.25. Between these two dates the insect had no opportunity of feeding. On dissection, *Herpetomonas* was found throughout the whole alimentary tract from the proboscis to the rectum. The upper part of the cardia was completely choked up by flagellates, large numbers being attached to the oesophageal valve and the epithelium of the cardia. The

oesophageal diverticulum contained large numbers of flagellates and was found contracting on an almost solid mass of flagellates immediately behind its junction with the oesophagus. The extent of the infection in this insect may be gauged by the fact that a smear made from the upper part of the cardia (including the oesophageal valve), which was dissected away from the remainder of the midgut after examination in the fresh, showed thousands of flagellates.

Every type of *Herpetomonas*, except the very thin, long forms, was found. This was the only instance where multiple division forms were found in a sandfly.

Material from this case was inoculated into two scarified points on the left forearm of a volunteer (Mr. M. Ber, of Jerusalem), to whom we tender our sincerest thanks.

27.II.25. The patient noticed two papules on the site of the inoculated points. His attention was drawn to them on account of itching. He was examined 29.II.25, and two papules, one about 0.7 mm. and the other about 4 mm. in diameter were noted (Pl. XVII, fig. 1). Smears from both showed numerous Leishman-Donovan bodies. Dr. Dostrowsky kindly examined the papules and stated that, clinically, the case (although suspicious) did not resemble a typical oriental sore. The papules gradually increased in diameter and scaling commenced. On the 17th of January, 1926, one papule was 1.8 cms. and the other 1 cm. in diameter. They were both covered with scales and the centre of each was covered by a scab (Pl. XVII, fig. 2). Dr. Dostrowsky again examined the patient and stated that the lesions differed from typical oriental sores in that the infiltration was not marked. Cultures were obtained on Kligler's modification of Noguchi's medium.

On February 8th the scales fell from the centre of each lesion; and marked infiltration round the centre of the lesion was present. Dr. Dostrowsky examined the patient and stated that, clinically, they were typical oriental sores (Pl. XVII, fig. 3).

#### THE ARTIFICIAL INFECTION OF *P. PAPATASII* WITH *HERPETOMONAS TROPICA* FROM ORIENTAL SORES

The object of the following experiments was two-fold, viz., to determine if *H. tropica* is capable of developing in *P. papatasii* after a feed on an oriental sore, and to find by direct experiment whether *H. tropica*, in passing through the sandfly, remains infective to man, i.e., whether man himself is the reservoir of cutaneous Leishmaniasis.

The aetiology of oriental sore in the light of the *Phlebotomus* theory (which is proved) is still obscure because of the absence or rarity of locally acquired cases in localities where sandflies are plentiful and cases from endemic foci are constantly present. The authors (1925) supposed that a third factor, apart from sandflies and human cases, was necessary to explain the curious distribution of cutaneous Leishmaniasis in Palestine, and particularly its absence from Jerusalem, but recently Dr. A. Dostrowsky, of the Rothschild Hospital, found a case of cutaneous Leishmaniasis in a child of seven who had never left Jerusalem.

The Sergeants, Lemaire G. and Senevet G. (1915), suspected lizards of being the reservoir of *Herpetomonas tropica*, and succeeded in obtaining cultures of *Herpetomonas* from the blood of the gecko (*Tarentola mauritanica*). Wenyon (1921) found *Herpetomonas* attached to the epithelium of the mucosa of the cloaca of *Chameleo vulgaris* in Egypt, and the authors found a specimen of *Acanthodactylus syriacus* from Binyaminah, near Haifa (a locality which is free from oriental sore), in which the small intestine contained numerous *Herpetomonas*, but no Leishman-Donovan bodies or *Herpetomonas* were found in the blood stream or in smears of the internal organs. The possibility of sandflies becoming infected with *Herpetomonas*, by feeding on lizards, or by the larva swallowing faeces of lizards, must be borne in mind, although the lizard theory also fails to explain the distribution of oriental sore.

It was decided, before dealing with lizards, to attempt to transmit cutaneous Leishmaniasis to man from sandflies infected by feeding on oriental sores.

Two cases were finally persuaded into submitting to feeding experiments with sandflies. One was a case of an ulcerated oriental sore acquired in Baghdad, and the other a non-ulcerating lesion acquired in Bethlehem. Both were particularly suitable for the purpose of the experiment, for simple blood smears made from the indurated margin of the lesions showed numerous parasites both free and intracellular. In such cases, on histological examination, parasites are found not only free and in the endothelial cells and leucocytes, but also in some of the endothelial cells lining the capillaries and small vessels of the affected area. In both cases parasites were so numerous that failure to infect any particular sandfly that fed on the lesion could not be due to the fact that the insect did not ingest Leishman-Donovan bodies with the feed.

The feeding experiments were conducted between 25.9.25 and 16.11.25, after which date both cases refused to submit to further experiments. Four hundred females, *Phlebotomus papatasi*, were given an opportunity of feeding on the lesions, and of these only one hundred and sixty-eight fed. The sandflies used for the experiments were not laboratory bred but were collected in Jerusalem, Mozzah (near Jerusalem), and Haifa, but in view of the fact that the infection rate of sandflies in Jericho, an endemic centre of cutaneous Leishmaniasis, was one in a thousand, and that the controls dissected from Jerusalem, Mozzah and Haifa were all negative, there is justification for considering that the infections subsequently noted in some of the sandflies were acquired as a result of the experimental feeds.

The sandflies used for the experiments were placed in specimen tubes three-quarters of an inch wide and two and a half inches deep, and three sandflies at a time were used for each feeding experiment ; (if more were placed in a tube they disturbed each other and refused to feed). Each batch of sandflies was allowed from ten to fifteen minutes for a feed ; it was found that if they refused to feed within fifteen minutes they refused to feed for the day. In several instances it was noted that an undisturbed sandfly interrupted its feed for a few minutes and then returned, made a fresh wound and completed its feed. The sandflies fed on the indurated skin not less readily than on normal skin. After feeding, the sandflies were transferred to test-tubes which were then closed with lightly-moistened cotton wool, and placed in a horizontal position (the wool must be moistened daily but no fluid must be allowed to enter the tube, for if the wings of *Phlebotomus papatasi* are even slightly moistened the insects adhere to the side of the tube and die). After the experimental feed the insects were allowed no further opportunities of feeding.

Of the total number of sandflies which fed on the lesions only sixteen were afterwards found to be infected with *Herpetomonas*, in spite of the fact that all or nearly all the insects must have ingested Leishman-Donovan bodies.

(Apart from the sandflies which fed on the two naturally infected cases of oriental sore, fourteen were fed on Case 1 of the experimentally transmitted cutaneous Leishmaniasis ; all fourteen proved to be negative on dissection a varying number of days after the feed, but owing to the small size of the lesion and the difficulty of manoeuvring the insect to feed on the actual minute site of the

lesion, the experiments were discontinued. By the time the second case of experimentally transmitted cutaneous Leishmaniasis appeared favourable for experiments no sandflies were available.)

The table on page 185 gives the data of each infected sandfly as observed during examination of freshly-dissected material.

The following experiments were performed immediately after the examination of fresh preparations in saline.

#### EXPERIMENT NO. 4

18.10.25. The whole of the midgut and hindgut of sandfly No. 39 was placed into a small pocket of skin scratched into the left forearm of a volunteer. Owing to the fact that the midgut of *P. papatasi* female contains a powerful anti-coagulin, the wound was observed continuously for three-quarters of an hour, till a solid clot was formed, in order to make certain that the inoculated material was not carried away with the escaping blood.

#### EXPERIMENT NO. 5

30.10.25. Material from sandfly No. 79 was inoculated into two scarified points on the left forearm of a volunteer. (The material for one inoculation was obtained by dissecting off the upper part of the cardia, including the oesophageal valve—this part contained about twenty flagellates. The other point was inoculated with saline containing flagellates removed with a fine capillary from the slide on which the dissection was made.)

#### EXPERIMENT NO. 6

3.11.25. Material from sandfly No. 127 was inoculated into two scarified points on the left forearm of a volunteer.

#### EXPERIMENT NO. 7

4.11.25. Material from sandfly No. 128 was inoculated into three scarified points on the left calf of a volunteer.

#### EXPERIMENT NO. 8

5.11.25. Material from sandfly No. 154 was inoculated into one scarified point on the left forearm of a volunteer.

Number	Origin of sandfly	Origin of sore on which sandfly fed	Date of feed	Date of dissection	Remarks
24	Jerusalem	Baghdad	30.9.25	3.10.25	Sandfly found dead. Five flagellates found in the stomach, four thick spindle forms and one long spirochaetal form.
39	Jerusalem	Baghdad	11.10.25	18.10.25	Midgut swarming with flagellates. Many attached to the oesophageal valve and to the epithelium of the cardia. Flagellates also present in the hindgut. No flagellates in the oesophageal diverticulum or anterior to the midgut. The majority of the flagellates were of the long thick type.
50	Haifa	Baghdad	25.10.25	28.10.25	Flagellates in stomach. All dead.
58	Mozzah	Baghdad	25.10.25	28.10.25	Numerous flagellates in midgut, particularly in stomach. None attached to the valve or the epithelium of the cardia. Short thick flagellates found and some short and very thin flagellates. A few intermediate forms.
79	Mozzah	Baghdad	25.10.25	30.10.25	Flagellates in midgut, mostly in the cardia. Some attached to the valve and upper part of cardia. Most of the flagellates of the long thick type.
83	Mozzah	Baghdad	25.10.25	30.10.25	Thick, medium-sized flagellates in midgut. None attached to the valve or cardia.
86	Mozzah	Baghdad	26.10.25	30.10.25	Long thick forms attached to epithelium of cardia. Long spirochaetal forms in stomach.
127	Mozzah	Bethlehem	1.11.25	3.11.25	Stomach swarming with flagellates. Some flagellates in cardia but not attached to epithelium.
128	Mozzah	Bethlehem	1.11.25	3.11.25	Stomach swarming with flagellates. Flagellates also present in the cardia. None attached to valve or epithelium of cardia. Flagellates seen passing into oesophagus and from oesophagus into pharynx. None in the oesophageal diverticulum.
133	Mozzah	Bethlehem	2.11.25	4.11.25	Heavy infection in stomach and cardia. Flagellates found attached to epithelium of cardia and others in process of attachment, the flagellae boring into the epithelium.
140	Mozzah	Baghdad	1.11.25	4.11.25	Slight infection in cardia only. Some attached to valve and to epithelium of the cardia, others in process of attachment.
143	Mozzah	Bethlehem	2.11.25	5.11.25	Slight infection (twelve flagellates seen) with long, thick flagellates in upper part of cardia. A few parasites attached to valve, others to epithelium of cardia.
147	Mozzah	Bethlehem	1.11.25	5.11.25	A few long thick flagellates in the cardia.
154	Mozzah	Baghdad	30.10.25	5.11.25	Whole of midgut swarming with flagellates. Clumps of flagellates attached to valve and epithelium of cardia. Long, thick flagellates. Type 3 of the a-flagellar forms and Leishmaniform bodies found in a smear of the uppermost part of the cardia, including the valve.
159	Mozzah	Bethlehem	1.11.25	6.11.25	Flagellates on the whole of midgut in oesophagus, oesophageal diverticulum, hindgut. The upper part of cardia almost choked up with flagellates. Many attached to valve and epithelium of cardia. Flagellates nearly all long, thick forms.
160	Mozzah	Bethlehem	1.11.25	6.11.25	Midgut swarming with flagellates, particularly cardia. Upper part of cardia almost completely choked up with parasites. Many attached to valve and epithelium of cardia. Flagellates also present in hindgut in oesophageal diverticulum, in oesophagus, pharynx, buccal cavity and proboscis. In proboscis flagellates are coiled up in chains. Some of them apparently dead. Type 3 of the a-flagellar forms found in a smear.



## EXPERIMENT NO. 9

6.II.25. Material from sandfly No. 159 was inoculated into one scarified point on the left forearm of a volunteer.

## EXPERIMENT NO. 10

6.II.25. Material from sandfly No. 160 was inoculated into one scarified point on the left forearm of a volunteer.

Thus a total of seven volunteers were used for the above experiments and a total of eleven inoculations were made ; of these, five were made from flagellates which had developed two days in the sandflies, four from flagellates which had developed five days in sandflies, one from flagellates which had developed six days in a sandfly and one from flagellates which had developed seven days in a sandfly.

Material from the sandflies not used for transmission experiments was fixed in absolute alcohol and stained with Giemsa ; the material left on the slides after the transmission experiments was also thus treated.

The feeding experiments recorded above are not sufficient to determine the whole cycle of development because none of the positive sandflies were examined before the second day after the feed, and thus the actual development of Leishman-Donovan bodies into *Herpetomonas* was not observed and no infection was found in sandflies dissected after the seventh day.

Examination of stained smears revealed the following facts.

1. Leishman-Donovan bodies, some of them showing stages of division, are found up to three days after a feed, i.e., at a time when there are already varieties among the flagellar forms. Some of the Leishman-Donovan bodies found were elongated and much bigger and thicker than some of the flagellar forms, and distinctly longer (length up to  $10\mu$ ) than any parasites found in the original skin lesion. There is, therefore, evidence of differentiation even before exflagellation takes place.

2. Two days after a feed, very short, thin flagellates (type 1 of the natural infections), thick, spindle-shaped or round flagellates (type 4), intermediate forms (type 7), and the long, very thin

flagellates in which the body is scarcely thicker than the flagellum (type 2) are present.

3. The long, very thin forms are present up to and including the fourth day.

4. After the third day the long, thick forms (type 6) appear and tend to become the dominant type, but the other forms (except the very long, thin forms), particularly those previously referred to as intermediate forms, are present.

5. On the fifth day schizogony forms (a-flagellar) appear (found in Nos. 154 and 160, in No. 154 in a smear of the upper part of the cardia) and Leishmaniform bodies were found in one instance (in No. 154, in a smear from the upper part of the cardia). These forms were very scarce. Multiple division forms such as those described from one naturally infected sandfly were not observed. Combining the examinations of fresh and stained material we may draw the following conclusions.

The ingested parasites exflagellate in the stomach and there they develop and multiply by division during the first three days. Subsequently many of them pass into the cardia, where some attach themselves to the epithelium of the latter and to the oesophageal valve by their flagellae. After the third day development and multiplication take place mainly in the cardia, particularly in the upper part, which may be completely choked up by parasites.

Flagellates pass into the oesophagus, oesophageal diverticulum, pharynx, buccal cavity and proboscis in the canal formed, when the mouth parts are at rest, by the under surface of the epipharynx and the upper surface of one mandible.

Taking these facts into account, and bearing in mind that there is an interval between the commencement of biting and the entrance of blood into the food canal, there is strong evidence for the view that *Herpetomonas tropica* is introduced into the skin by the bite of *Phlebotomus papatasi*. It is true that some of the flagellates found coiled up in the proboscis were dead, and that the proboscis is not a suitable habitat for the flagellates, but since active division and development take place in the uppermost part of the midgut, from where flagellates may continually pass forward into the oesophagus and pharynx and thence to the buccal cavity and proboscis, the dead flagellates in the proboscis can be continually replaced by living ones.

The fact that *Phlebotomus papatasi* only very exceptionally passes fluid per rectum during the act of feeding further supports the above view. (*H. tropica* may further enter the skin by the crushing of an infected sandfly.)

The volunteers inoculated from the artificially infected sandflies were observed until 18.2.26, and the result in each case was negative. This surprising result may be due to the facts that :—

1. The observation period is not sufficiently long. This cannot account for all the negative results in view of the relatively short incubation periods in the two positive cases from naturally infected sandflies.

2. The parasite, in passing from man to the sandfly, becomes non-infective for man, i.e., man is not the reservoir of *Herpetomonas tropica*.

3. The end point of the biological development of *Herpetomonas tropica* in *Phlebotomus papatasi*, whereby infective forms are produced, was not reached. This view gains support from the fact that the variety of forms noted in one instance, of a naturally infected sandfly, material from which gave such striking results in Experiment No. 3, was not found in the artificially infected sandflies.

The authors are inclined to the last view, but a discussion would only be of theoretical interest at present, and the matter must be decided by further observations and experiments.

#### SUMMARY AND CONCLUSIONS

1. The infection rate of sandflies with *Herpetomonas* in Jericho was found to be about one per thousand during 1925.

2. The morphology of naturally occurring *Herpetomonas* in *Phlebotomus papatasi* is described.

3. Two out of three experiments in transmission of cutaneous Leishmaniasis from sandflies naturally infected with *Herpetomonas* were successful.

4. Sandflies were artificially infected with *Herpetomonas tropica* by feeding on oriental sores, 10 per cent. of the sandflies being infected.

5. Attempts to transmit oriental sore to seven volunteers, by eleven inoculations with material from sandflies artificially infected with *Herpetomonas tropica* were all negative within an observation period of three and a half to four months.

## ADDENDUM

The volunteer used for Experiment No. 2 and examined on December 15th, 1925, with a negative result left Palestine for leave in Europe. He returned to Palestine on the 24th March, 1926, and was again examined. A hard nodule was found in the subcutaneous tissue beneath the site of one of the inoculated points. The nodule was not attached to the skin, which was perfectly normal, or to the deep tissues, and was freely movable. The patient first noted the nodule in January, 1926. The nodule grew rapidly in size, and by April 14th, 1926, was about 9 mm. long and 6 mm. broad, and still remained freely movable. The patient suffered no pain or inconvenience from the presence of the lesion.

On April 14th, 1926, the nodule was punctured and Leishman-Donovan bodies were found. (Cultures made on a modification of Noguchi's leptospira medium on 22.4.26 were examined 28.4.26 and found positive.)

The case was seen by Dr. A. Dostrowsky, dermatologist to the Rothschild Hospital, who stated that out of over a hundred cases of oriental sore examined by him in Palestine none were observed with such a lesion.

On April 20th, 1926, two more volunteers were each given two inoculations from puncture fluid from the lesion of the above-described case. In one case the inoculations were made intracutaneously, and in the other subcutaneously, in order to determine whether the lesion produced in Experiment No. 2 was specific or due to a deep inoculation of the flagellates.

The results will be recorded in a future communication.

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## EXPLANATION OF PLATE XVI

Morphology of *Herpetomonas* in *Phlebotomus papatasi*.

- Figs. 1 to 11. Leishmaniform bodies.  
     1 to 8, from a naturally infected sandfly.  
     9 to 11, from an artificially infected sandfly.  
     5 to 8, from a smear of the upper part of the cardia.
- Figs. 12 to 13. Leishmaniform bodies with rhizoplasts. Natural infection. From a smear of upper part of cardia.
- Figs. 14 to 25. Showing a-flagellar schizogony forms.  
     14, large round form with a single nucleus and blepharoplast.  
     15 to 17, a single nucleus and a number of blepharoplasts.  
     18 to 25, a varying number of nuclei and blepharoplasts.  
     19, 20, 21, 23, from artificially infected sandflies.
- Figs. 26 to 28. Small pyriform parasites with a short flagellum.  
     26 and 28, from an artificially infected sandfly.
- Figs. 29 to 38. Larger, round and spindle-shaped flagellates.  
     29, 31, 33, 34, from an artificially infected sandfly.  
     37 and 38, dividing.
- Fig. 39. Irregular form with numerous vacuoles and granules in the protoplasm.
- Figs. 40 to 45. Short, thin forms.  
     40, 42, 43, from artificially infected sandflies.
- Figs. 46 to 48. Long, thin forms.  
     47 and 48, from artificially infected sandflies.
- Figs. 49 to 52. Types of dividing forms.  
     49 to 51, from artificially infected sandflies.  
     52, appears to be an abnormal dividing form.
- Figs. 53 to 64. Intermediate forms.  
     53, 54, 55, 56, 58, 59, 60 and 63, from artificially infected sandflies.
- Figs. 65 to 71. Long, thick forms. All from artificially infected sandflies.  
     70 and 71 (semi-diagrammatic), from proboscis.
- Figs. 72 to 75. Multiple division forms. From a smear of the upper part of the cardia. All from a naturally infected sandfly.
- Fig. 76. Cluster of flagellates. Camera lucida drawings  $\times 1200$ , except 70 and 71.









## EXPLANATION OF PLATE XVII

Development of cutaneous Leishmaniasis on upper part of forearm.

Experiment 3. (Inoculation from naturally infected sandfly,  
on 2.11.25.)

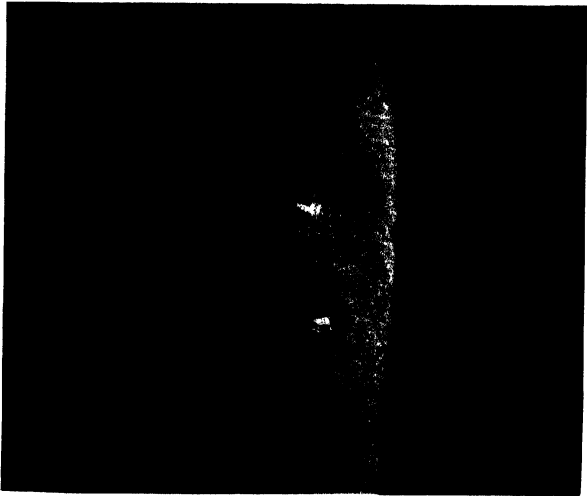
Fig. 1. Papules about two-thirds of natural size.

Fig. 2. Natural size, 17.1.25.

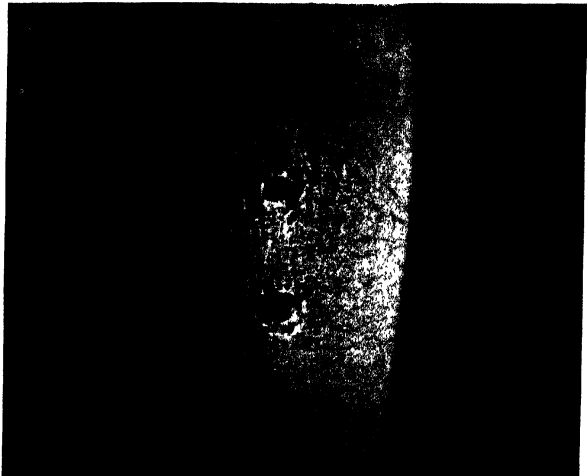
Fig. 3. Natural size, 11.2.26. (Slightly different view from 2.)



I.



2.



## EXPLANATION OF PLATE XVIII

- Fig. 1. Mass of flagellates attached to uppermost part of cardia, including oesophageal valve. Artificial infection after five days. Photograph of fresh dissection.  $\times 220$  (*circ.*).  
S.G.—Salivary gland.  
O.D.—Oesophageal diverticulum.  
M.M.—Solid mass of flagellates.
- Fig. 2. *Herpetomonas* attached to epithelial cells of cardia, natural infection. Photograph from stained preparation.  $\times 850$ .
- Fig. 3. *Herpetomonas* attached to oesophageal valve, natural infection. Photograph of fresh preparation.  $\times 220$  (*circ.*).



1.



2.





# A CASE OF CEREBRO-SPINAL MENINGITIS

BY

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*(Received for publication 25 March, 1926)*

The isolation of the meningococcus in Trinidad, and its apparent association with a pathological condition in the ear, render the following case of some interest.

Patient, male, *aet.* 47, admitted to Hospital on 17th November, 1925, with pain, tenderness and swelling over right mastoid, and a history of headache and pain and discharge from right ear for two weeks, temperature normal. With local treatment the discharge lessened, but the headache increased in intensity. Temperature continued normal until the morning of 26th, when it rose to 101-103° F., with obvious signs of meningitis, e.g., vomiting, convulsions, coma, rigidity of muscles of back and neck, strabismus, and Kernig's sign. The pupils were dilated. Lumbar puncture was at once performed and about 30 c.c. fluid withdrawn. The discharge from the ear was, unfortunately, not submitted to bacteriological examination. There was no discharge from the urethra. Death occurred at 2.20 p.m. on 27th.

The cerebro-spinal fluid escaped under pressure. It was turbid and purulent and contained globulin and albumin. Smears made from the centrifugalized deposit stained with methylene blue and by Gram's method showed numerous leucocytes—polynuclears 90 per cent.—and small bean-shaped gram-negative diplococci arranged in pairs or, occasionally, in fours, both intra- and extra-cellularly. Cultures were made, half-an-hour after withdrawal, on ordinary agar, 2 per cent. glucose hydrocele fluid agar, 'vitamin' blood agar and Gordon's trypsin legumin agar. There was no growth on ordinary agar in forty-eight hours. On glucose hydrocele agar typical small, grayish, finely granular, viscid, discrete colonies were seen, on 'vitamin' blood agar, in twenty-four hours, the colonies were somewhat larger with a ground glass appearance. On Gordon's

trypsin legumin agar they were circular, moist, greyish and translucent. Stained smears from these cultures showed gram-negative cocci with the morphological appearance of the meningococcus. Through the lack of agglutinating sera serological tests were, unfortunately, not done. Subcultures were made into glucose, lactose, mannite, laevulose and saccharose ; glucose and lactose were alone fermented, giving the sugar reactions characteristic of the meningococcus.

*Post Mortem*, permitted only on the head, revealed a basal purulent meningitis, extending to the Pons and Cerebellum and into the Sylvian fissures, with the blood vessels greatly engorged with blood. The lateral ventricles were distended. There was no cerebral abscess, no localization of the exudate to the right side, and no evidence of lateral sinus septic thrombosis. The exudate at the base of the brain showed gram-negative diplococci. The spinal fluid was also turbid and purulent and the exudate was seen extending into the cervical region. Sections made through the mastoid showed a few cells in the upper part with a brownish purulent material which *also contained gram-negative, bean-shaped diplococci*. Cultures from this material on the above media proved negative.

The case seemed to have been one of otitis media with cerebro-spinal meningitis, both due to the meningococcus. Stitt mentions that 'the meningococcus may cause otitis media.' Park and Williams state that 'the finding of gram-negative cocci in the cerebro-spinal fluid either intra- or extra-cellularly is presumptive evidence of meningococcal meningitis,' and Muir and Ritchie that 'the presence of gram-negative cocci, especially within the cells, is practically diagnostic of a case of cerebro-spinal meningitis'; such also is the opinion of Gaskell, Treadgold and Arkwright, as reported by the Medical Research Committee.

# A CASE OF *GIARDIA INTESTINALIS* TREATED WITH STOVARSOL

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Child, male, *aet.* 2½ years, refused its usual meals on 13th December, 1925. At about 9 p.m. that day he was seized with severe abdominal pains, 'doubling up' of the lower extremities upon the abdomen, and retraction of the head with the passage of twelve stools in twelve hours. At 9 a.m. on the 14th, temperature was 104° F., the abdominal pains were evidently very intense and the child looked very ill. A mild purgative and rectal lavages, etc., were given, and the stools submitted to microscopical examination showed active flagellate forms of *Giardia intestinalis*. From 9 a.m. to 9 p.m. that day, twenty to twenty-four motions were passed. They were watery and of a bright greenish colour, 'like masses of floating sea moss.' The child was given one-sixth grain emetine subcutaneously, on the 15th another one-sixth grain, and on the 16th and 17th, one-quarter grain each day with rectal lavages, etc.; with only slight improvement, the temperature falling to 101° F. The stools, however, still remained frequent without any evidence of the formation of faeculent matter. On the 18th one grain of stovarsol was given by the mouth, and the emetine stopped. Twenty-four hours later the stools became less frequent and showed signs of becoming formed, the abdominal pains were less severe, the child seemed less distressed and the temperature was normal. One grain of Stovarsol was given on each of the three following days, at the end of which time the motions were faeculent and formed, all pains had disappeared and the temperature continued normal. Examination of the stools, however, still revealed the presence of *Giardia*. The Stovarsol was then given every other day for six days, at the end of which time examination of the stools, which were then normal, showed no *Giardia*. The Stovarsol was continued, one grain every third day for fourteen days, and further



examination of the stools showed no *Giardia*. The child has since remained quite well.

Dobell states that emetine has no effect whatsoever upon *Giardia intestinalis* or any of the other 'common intestinal flagellates,' nor has methylene blue, as advocated by Castellani. Oral administration of Salvarsan is said to have cured cases in human beings, by Bavant, Carr and Chandler, and in mice and rats, by Rogers, Kofoed, Yakimoff and others, but Dobell finds the evidence for cure in these cases inconclusive. 'It appears probable,' he says, 'from the evidence at present available, that no specific treatment for infection with any species of intestinal flagellates has yet been discovered. When negative examinations are made during or after a course of "specific" treatment, they cannot be regarded as evidence of "cure" unless they extend over a period much longer than any "negative period" which may be observed in any untreated cases.'

In the above case examination of the faeces on 26th January, 1926 (i.e.) twenty-eight days after last 'negative' examination, still showed no *Giardia*.

# A CASE OF PYELITIS ASSOCIATED WITH *ENTAMOEBIA HISTOLYTICA*

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Patient F., *act.* 58, suffered from periodic attacks of dysentery with blood and mucus and *Entamoeba histolytica* in stools, relieved by emetine injection, last attack being in end of November, 1925. On December 27th, she showed signs of acute cystitis, e.g., frequent and painful micturition, burning sensation in urinary meatus, pain and tenderness over bladder, acid urine containing mucus and albumen. There was no vaginitis. The condition cleared up with the rest, urotropine, etc. On January 6th, 1926, patient developed sudden acute pain and tenderness in the left loin over the kidney region, with rigors and a temperature ranging from 104.8°F. to 101.4°F. She was treated with alkaline diuretic mixture and urotropine, but was only slightly relieved. The temperature persisted, being, on 12th January, 101° and 100.4°F. On that day the urine was examined microscopically. It showed pus, bladder and renal cells, and *Entamoeba histolytica* (vegetative form). A blood count showed Poly. = 91.9 per cent., Lympho. = 3.2 per cent., Eosin = 0 per cent., Large Mono. = 1.9 per cent., Myelocyte = 3.0 per cent. One-and-a-half grains of emetine was given subcutaneously on the morning of the 13th, and a catheter specimen of urine carefully taken. This also showed *Entamoeba histolytica*. Towards the evening of that day the temperature fell to 99° F., and the following morning (14th) it was 98.4° F., when a further half grain of emetine was given. The temperature remained normal and from the evening of the 14th all the symptoms cleared up. Patient has remained well since. Repeated examination of urine showed no calculus and urine of 19th was normal and showed no entamoeba. Dobell states that Baelz (1883) was the first to describe

*E. histolytica* as occurring in the urine. Craig (1911), Fischer (1914), and Walton (1915) have also described cases. Castellani and Chalmers mention cases of amoebic pyelitis reported by Posner and Chalmers, and O'Farrell, and recognise the condition as a definite clinical and pathological entity.

I am indebted to Dr. V. M. Metivier for the clinical notes.

# A CASE OF INFECTION WITH *LAGOCHEILASCARIS MINOR* (LEIPER)

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Patient male, *aet.* 16, a native of Trinidad, always resident in the southern part of the island opposite the mainland of South America, was admitted to hospital on 4th August, 1924, with the following history. About three months previously he felt a hard, painless but slightly tender lump, size of a pea, near the angle of his *right* jaw. The lump increased to the size of a walnut and formed a small area of suppuration from which there escaped a yellowish-white purulent material. Two weeks later his *right* eye began to pain, it became congested and inflamed and a purulent discharge appeared.

When admitted to hospital there was seen a hard, painless, uniformly smooth tumour about the size of a tangerine, movable from side to side, below the angle of the right jaw, having a ragged, foul, sloughing, unhealthy ulcer, about the size of a shilling, in its centre, and showing a sharp irregular margin with four well-marked sinuses from which there escaped, on pressure, a thin purulent exudate containing numerous small worms 3 to 4 mm. long. The right eye showed a purulent conjunctivitis and iritis with considerable chemosis.

In spite of energetic treatment a severe panophthalmitis resulted, necessitating enucleation of the eye ten days later (14th). On the 21st the tumour mass was still hard and discharging, on pressure, matted coils of very actively moving worms.

On the 23rd, patient complained of pain in his *right* tonsil, which was congested and enlarged, showing some dirty yellow masses in its crypts. A swab taken for Klebs Löffler B. proved negative.

On 3rd September he complained of dyspnoea and examination of the heart revealed only an accentuated aortic second sound. Lungs were normal. On the 5th, under chloroform, the whole

tumour mass was removed, together with the enlarged glands in its immediate neighbourhood.

The resulting wound and the cavity of the empty orbit healed normally but his right tonsil, which had not subsided in size, became more painful on the 14th, and in spite of local treatment was seen, on the 22nd, to be very enlarged and ulcerated, with a foul sloughing surface. On the 26th, under chloroform, the tonsil was removed and showed itself practically a sac of pus containing, embedded in its substance, worms similar in appearance to those found in the cervical tumour. On the 6th October patient was discharged from hospital, well.

Specimens of the worms obtained from the tumour mass and the tonsil, transmitted to Professor Leiper, of the London School of Tropical Medicine and Hygiene, were identified as *Lagocheilascaris minor*, first described by him, in 1909, as occurring in cutaneous abscesses, in Trinidad, and since found, also by him, among specimens received from Dutch Guiana, in South America.

# THE FURTHER DEVELOPMENT OF *ONCHOCERCA VOLVULUS* LEUCKART IN *SIMULIUM DAMNOSUM* THEOB.

BY

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## PLATE XIX

In a previous paper Blacklock (1926) showed that the larval form of *Onchocerca volvulus* which can often be found in the skin of infected human beings, is frequently taken up by *Simulium damnosum* when feeding on such infected skin. The larvae so taken up can be found alive and active in the gut of the insect often in large numbers. It was further shown that progressive development of the larva goes on in the gut and thorax, a development which is signalized by changes of form and increase in size of the larva.

The present series of experiments was carried out in the Konno District of Sierra Leone during part of December, 1925, and January and February, 1926, using wild flies and the same infected subject as before, the 'Case 50' referred to in the account of the former experiments. Six series of flies were allowed to feed on this case; as the early stages of the larval development had already been studied very few dissections of flies were made in the first days immediately following the infecting meal. Attention was rather directed to obtaining again those more advanced stages of the development which were described as occurring in some of the flies in the previous experiments, and to ascertaining whether developmental stages beyond this could be found such as would justify the conclusion that this species of *Simulium* can actually transmit *O. volvulus* infection. As a result of the additional work on the subject it is now possible to add considerably to the facts recorded

in the above paper. The present experiments have not only confirmed fully the conclusions therein reached, but have also elicited evidence that the development proceeds regularly until the head of the fly is invaded by the developing worms. Not only so, but it has been possible to demonstrate that, given favourable circumstances, these fully developed cephalic forms readily escape in numbers from the anterior part of the head in the proboscis region. Although the attainment of this degree of development might in itself be accepted as conclusive evidence that the fly concerned is capable of transmitting a filarial disease, final proof of this has been sought by injecting intra- and sub-cutaneously into two *Cercopithecus* monkeys the advanced forms of larvae in the head of *Simulium*.

EXPERIMENT I. On December 28th, 1925, from 7.30 to 9 a.m. 42 flies were caught when feeding on Case 50 from the waist down.

EXPERIMENT II. On December 29th, from 6 to 9.30 a.m. 67 flies were caught feeding on any part of the body.

EXPERIMENT III. On December 30th, from 8 to 10 a.m. 41 flies were caught feeding on any part.

EXPERIMENT IV. On January 16th, 1926, from 8 to 10.30 a.m. 117 flies were caught feeding on any part.

EXPERIMENT V. On January 26th, from 8 to 10 a.m. and 4 to 6 p.m. 110 flies were caught feeding on any part.

EXPERIMENT VI. On February 16th, from 4 to 6 p.m. and on February 17th, from 6 to 8 a.m., and from 4 to 6 p.m. 125 flies were caught feeding on any part.

Owing to enforced absence for part of the time immediately following capture of the flies in Experiment VI, the records of death of flies up to the seventh day could not be kept; they are, therefore, omitted from the table showing the length of life of the flies after capture. The parasitic findings in those flies of this experiment which survived for this period and were subsequently dissected are, however, included in the table of dissections.

It will be seen that only in Experiment I was any restriction imposed on the flies as to which portion of the body they should bite. The subsequent experiments involved no such restriction, since it was hoped that in this way certain of the flies would obtain light infections which would permit of their surviving longer. It

was found, however, that when the patient was seated close to the ground, raised only a few inches off it, by far the majority of the flies fed on the region of the waist and downwards.

The length of life, infection rates, and ovarian development of the flies are set out in the following tables.

### *Length of Life.*

TABLE I.

Showing the number of flies in five experiments dying on each day after the infective feed.

Number of experiment	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Lost	Killed for dissection
I	Number of flies 42	6	3	2	6	2	2	1	2	4	3	6	2	2	1	...	...	...	...	...	0	0
II	67	11	4	1	5	4	4	4	11	2	1	5	11	3	0	0	0	0	0	1	0	0
III	41	8	1	3	2	3	0	1	0	3	7	10	1	0	*0	0	1	...	...	...	*1	0
IV	117	17	6	12	10	6	16	17	19	15	1	...	...	...	...	...	...	...	...	...	...	13 115
V	110	24	14	9	10	5	6	8	6	9	13	6	...	...	...	...	...	...	...	...	...	...
Totals...	377	66	28	27	33	20	28	31	28	23	25	27	14	5	1	0	1	0	0	1	1	18

It is seen from Table I that no fly survived for a longer period than 19 days, although it is possible that some of those flies killed for dissection might have lived longer; it is not without interest that the three flies which actually lived for the longest time, 19, 16, and 14 days respectively, had apparently not fed on infected skin or on blood, as infection was not present and the ova were not developed. The highest mortality among the flies on any single day was usually on the first day after capture, due possibly in part to some mechanical injury during feeding. As regards the mortality which occurs in the first week or so, if we exclude the effects of actual confinement and fungus infections which readily attacked the flies, the most important causes of mortality appeared to be hyperinfection of the fly with larvae, and the disturbance of metabolism produced by development and retention of ova. A third cause or group of causes is doubtless the influence of such factors as temperature and humidity. But in infected flies fed on blood the influence of these



last factors is so complicated by the resultant changes produced by them on ovarian and parasitic development that it is difficult to determine what influence they really have. For example, it became evident, as will be shown presently, that a low temperature affected adversely not only the development of the ovaries of the insect, but also that of larvae ingested. The effect of this retardation should therefore be to prolong the life of the infected fly; but there is some evidence that even apart from the parasitic and ovarian development, low temperature may have a deleterious effect on the fly. Some flies which had not absorbed sufficient blood to promote development of the ovaries and which had not obtained infection with larvae from the skin, died of apparently no other cause than cold. The possibility of this being the case is not excluded by the fact already referred to that the flies which lived longest after feeding happened to be uninfected and apparently had not fed on blood.

*Infection of SIMULIUM DAMNOSUM with larvae of O. VOLVULUS.*

In Table II are given the total numbers of flies used in each experiment, the total numbers dissected after the third day, the number found infected and the site of infection in the fly.

TABLE II.  
Showing results of dissection of fed flies.

Number of experiment	Total flies	Dissected	Negative	Positive	Developmental forms found in	
					Thorax	Head
I	42	21	8	13	13	2
II	67	41	12	29	28	1
III	41	20	5	15	15	2
IV	117	60	18	42	33	17
V	110	29	8	21	20	6
VI	125	30	9	21	18	6
	502	201	60	141	127	34

It is seen from Table II that of 201 flies which were dissected at a period later than the third day after feeding, 127, that is 63 per cent., showed thoracic infection, while 34, that is 17 per cent., were infected in the head.

### *Head infection.*

In the different experiments there was considerable variation not only in the rapidity of occurrence and the proportion of head infections but also in the actual number of larvae found in this region. The occurrence of infection of the head was considered established ; when on separating the head from the thorax larval forms were found protruding from or emerging from the cut posterior end of the head ; when such forms emerged from the proboscis region before or during separation of the head or were found by dissection of the tissues of the head after it had been separated ; when larvae emerged from the proboscis region spontaneously.

### *Lapse of time between infective feed and head infection.*

The earliest day on which larvae were found in the posterior part of the head was, in the case of Experiment IV, Fly 51, five days after the infecting feed ; the earliest day on which larvae were found in the anterior portion of the head was seven days after the infecting feed. Of the 34 flies which became infected in the head, twelve had posterior infection, seventeen anterior infection, while nine had infection in the mid-region of the head. The figure for the latter region is lower than it should be because several heads which had proved infected by the escape of larvae either anteriorly or posteriorly were preserved for sectioning or used for injection and therefore were not included in the dissections in so far as the mid-head was concerned.

### *Temperature in relation to development of larvae in the fly.*

Notable differences in the number of head infections obtained in different experiments are accounted for largely by the temperature conditions. For example, in the first three experiments, totalling 150 flies, only five infections of the head were discovered ; yet 78 of the flies survived for a period of six days or over and were dissected, and as we have seen posterior infection of the head can occur as early as five days after feeding in favourable conditions. In the fourth experiment, with a total of 117 flies, no less than seventeen infections of the head were discovered ; yet only 66 of these flies survived for a period of six days or over and were dissected.

The first three experiments were carried out in an unwallled building at the time when the cold Harmattan wind was blowing.

The absolute maximum temperature recorded was 88, the absolute minimum 49, while the average minimum during the period was 58. The fourth experiment was carried out at a time when the external temperature was much higher, and moreover the flies were not kept in the open building but in a walled building where the temperatures recorded for the period were :—absolute maximum 87, absolute minimum 63, average minimum 68. This experiment was therefore carried out at an average minimum temperature ten degrees higher than that of the first three experiments. The fifth experiment stands intermediate in average minimum temperature and also in the resultant head infections. The influence of temperature in these experiments is shown in Table III.

TABLE III.

Giving the total and head infections in flies 6 days or more after the infective feed.

Number of experiment	Temperature			Flies 6 days or over dissected	Total infected	Percent-age	Infected in head	Percent-age infected in head
	Maximum	Minimum	Average minimum					
I, II, III	88	49	58	78	53	68	5	6
IV	87	63	68	52	37	71	16	31
V	84	60	63	29	21	72	6	21

It is seen that almost equal percentages of flies in the three sets were found to be infected at the time of dissection. Numerically speaking, therefore, development had been equal in all three sets. It is when we come to consider the degree of development that we are struck by the great differences which exist, especially between set one and set two. Here the retarding influence of low temperature on the development of the parasites is very evident ; the conclusion is supported by the facts shown in the third set. It is perhaps better to speak here of the retarding effect of low temperature, than of the accelerating effect of high temperature, because in point of fact the period during which anything approaching such low temperatures have been recorded in the Konno country is limited to those one or two months of the year when the Harmattan may be blowing.

*Development of ovaries and oviposition.*

In Table IV are given the numbers of flies in which notes on the condition of the ovaries were made and the occurrence of infection in relation to ovarian development.

TABLE IV.

Showing ovarian development and infection of flies.

Number of experiment	Ovaries dissected	Ova developed	Flies		Ova not developed	Flies	
			Infected	Not infected		Infected	Not infected
I	17	10	7	3	7	1	6
II	36	23	22	1	13	2	11
III	19	14	14	0	5	0	5
IV	57	44	40	4	13	1	12
V	29	23	21	2	6	0	6
VI	30	24	21	3	6	0	6
	188	138	125	13	50	4	46

It will be seen that though there is a somewhat close relationship between development of the ovaries and infection it is by no means absolute. A number of flies in which the ova were fully developed presented no infection. This condition could be brought about by the fly being resistant to infection or feeding on a portion of uninfected skin, so that while it would take up blood it would fail to take up any larvae.

*Oviposition in tubes.*

In experiment I, one fly was found on the ninth day to have oviposited on the glass tube; the egg mass, yellow in colour, tough and elastic in consistency, was then almost dry. There was a tendency to a linear arrangement of the eggs at the margin of the mass but the centre was several layers thick and irregular. In this case oviposition was incomplete and the fly was dead; this was the rule where oviposition occurred during the experiments; the fly would

be found glued by a wing or by the tip of its abdomen to a mass of eggs laid in a very haphazard manner and unless liberated at once the fly soon died ; in some cases a large number of eggs remained in the fly, while in others one or two would be found retained ; in Table V are given the figures for oviposition found in the first five experiments.

TABLE V.

Giving the dates on which oviposition occurred in tubes during the experiments.

Number of experiment	Total flies which oviposited	Earliest day after feeding and numbers laying	Latest day after feeding
I	1	9th (1)	0
II	0	...	...
III	0	...	...
IV	11	5th (3)	7th
V	2	10th (2)	...

From this table it is seen that if we group the first three experiments together as before, and compare them with number four, there appears a very pronounced difference not only with regard to the number of flies ovipositing but also in the length of time which elapses between the date of feeding and the date of oviposition. Taking the total flies it is found that of the 150 used in the first three experiments 0.6 per cent. oviposited, while of 117 used in the fourth experiment 9.0 per cent. oviposited. We have here evidence that low temperature has produced in the first three experiments a definite retardation of the ovarian development just as it does of the parasitic larval development.

#### *Relation between ovarian and parasitic development.*

From what has been said it is clear that several of the factors which influence the rapidity of development of the ovaries have also an influence on the rapidity of the development of the parasites in the insect host tissues. Especially interesting is the correspondence between the time when oviposition can occur and the time when the parasites can reach the head. For it is extremely probable, in view

of this correspondence in time of these two developmental cycles, that the fly, after having oviposited, will be in position to infect in the act of biting any individual on whom it feeds soon after oviposition.

*Emergence from the head of Simulium.*

*On slight pressure.* When the head was infected in the anterior region it was possible to see the larvae emerging into the fluid during the manipulation of the needles in separating the head from the thorax or by slight pressure on the separated head. Again, even before attempting to separate the head a slight pressure would sometimes make them escape.

*Spontaneous emergence.*

This interesting phenomenon was observed on three occasions, which deserve special consideration. When it was observed that head infection was present preparations were made in order to try and inoculate the head forms into monkeys. It was thought that if monkey serum was used as the fluid for dissecting purposes the larvae would have a better opportunity of infecting the animal than if other media were used. Fly No. 101 of experiment IV was killed by carbon tetrachloride and was placed beside a drop of 48-hour old monkey serum warmed to body temperature in a hollow ground slide. The slide was put under the dissecting microscope, and the fly was pushed towards the drop of serum till its head was lying in it. Before dissection of the head from the thorax could be commenced it was noticed that larvae began to emerge from the anterior part of the head into the serum of their own accord, first one, then two and three, until ten had emerged and were moving actively in the serum. The larvae came out smoothly with a gliding movement until about a tenth of the length remained in the head and then the last portion of the body was delivered more suddenly, as if expressed by muscular contraction. This observation was so remarkable that an endeavour was made to repeat it and also to check it by using saline solution instead of serum. Fly, number 108 was killed and placed in the same way with its head in monkey serum warmed to body temperature; after one minute, larvae began to emerge from the anterior part of the head. Fly 109 was killed and placed with its head in the edge of a drop of normal saline warmed to

body temperature. In five minutes' observation no larvae emerged ; the fly was then transferred to monkey serum and after the lapse of two minutes one larva emerged ; the head was separated and dissected, but no further larvae were discovered.

These experiments, few as they necessarily are, nevertheless indicate clearly that warm monkey serum exercises an attractive influence on those larvae which are present in the anterior cephalic region of the fly and are sufficiently advanced to be ready to emerge. A similar attractive influence is not exercised by warmed normal salt solution. The results suggest that the entrance of mature larvae into the skin or into a wound during the period when the insect is biting may be determined by quite other factors than mere mechanical coincidence. It is worth recalling here that all those flies from which the head forms of larvae emerged thus spontaneously into warmed monkey-serum had fed on raisin, but had not in so doing got rid of the larvae in the head, as would be expected if the injection of larvae resulted solely from the mechanical processes of biting.

### *Experimental inoculation.*

The following are the details of the attempts to infect monkeys with the larvae in the head of *Simulium*.

The first monkey received on 25.1.26 in the skin and subcutaneous tissue of the right flank the heads of six *Simulium damnosum*, Numbers 102 to 107 of experiment IV, on the ninth day after the infecting feed. The previous fly 101, dissected immediately before, had the head infection referred to above, and the next fly in the series, 108, had also head infection demonstrated by spontaneous emergence of larvae into monkey serum. In making the dissections of the heads which were infected many active larvae escaped into the fluid ; this fluid containing larvae was also rubbed on to the cut edge of the skin incision in the monkey's flank.

The second monkey received on 26.2.26 into the skin of the head over the left ear the heads of eight *Simulium damnosum*, of which three were known to be infected in the head and two more in the thorax. There was, however, some little difficulty with the insertion of them and one or two were lost, so that the chances of infection appear not quite so good in this animal. Neither animal

appeared the worse for the inoculation ; at the time of writing, that is, two months after the inoculation of the first monkey, there is no evidence of infection or nodule formation.

### *Proboscis infection.*

When larvae emerged from the proboscis they appeared at the level of the anterior margin of the labella usually to one or other side of the mid-line. They were never seen to emerge from the hypopharynx nor were they found on dissection in the salivary glands, the salivary ducts, or the common salivary duct. In flies which were fixed in alcohol at a time when larvae had begun to come out of the proboscis, and were then cleared in clove oil and mounted, larvae could be seen coiled up at the base of the labium, while others, in varying degrees of extension, could be seen reaching forward into the portion of the labium behind the labella. The labium of *Simulium damnosum* is a large structure, comprising anteriorly the thick fleshy labella and posteriorly the wide but thinner membranous portion. It is a soft, scantily chitinized organ which appears well-adapted by its structure to accommodate large larvae, and also capable, owing to the membranous nature of its walls, of providing an easy exit for them from the proboscis during the act of feeding.

### *Summary of developmental stages in the fly.*

In Fig. 1, A—E, are illustrated the main types of larval developmental stages found in the thorax and head. It is not to be understood that these are the only forms found because there are numerous minor modifications seen in the study of a series of dissections. Nor is it to be understood that each of the forms illustrated represents the only form seen on the day when this form may predominate. It was observed for example that even as late as the seventh day forms were occasionally found which resembled the primary gut forms ; though rather larger in dimensions and paler staining than normal their state of preservation appeared so good that one could only conclude that although they had not developed yet they had survived for many days. In the same way in dissecting the thorax it was not unusual to find forms representing very different stages of development in the same thorax.



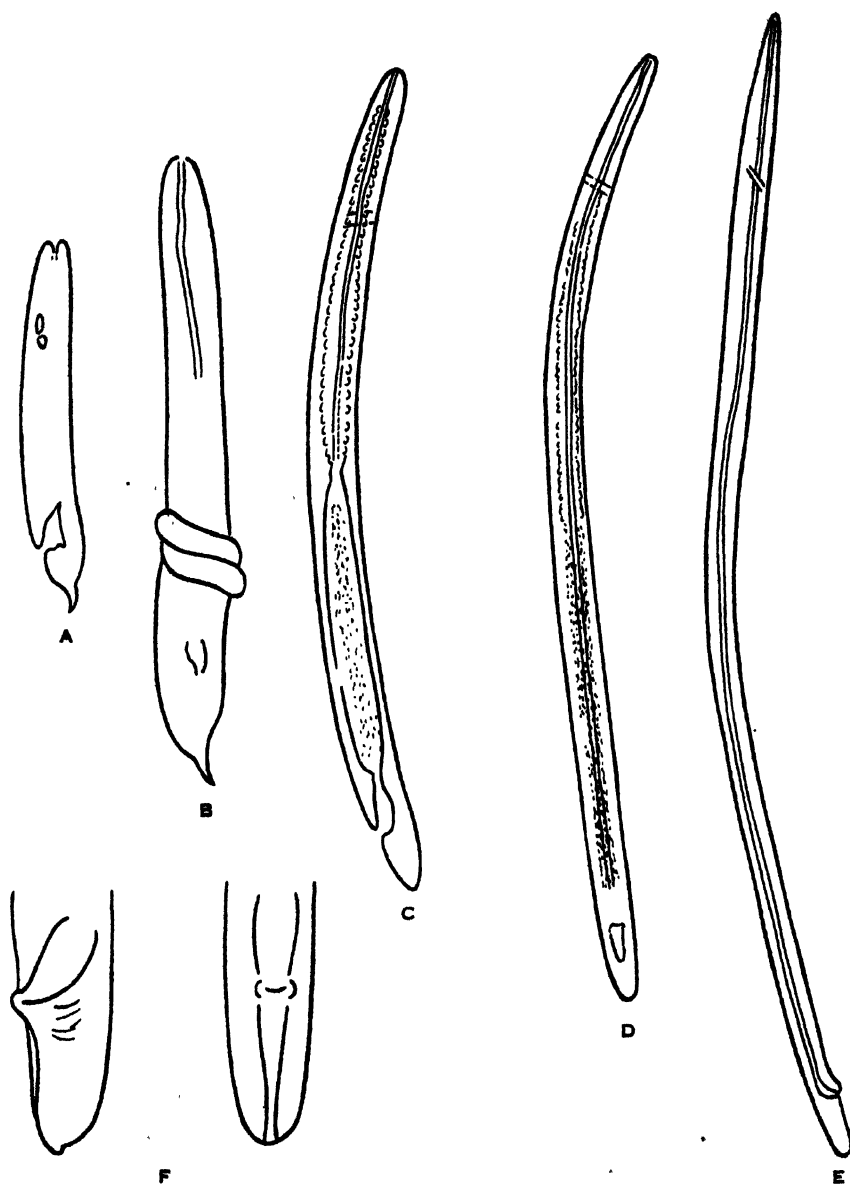


FIG. 1. *A*.—Early thoracic form, second day. *B*.—Thoracic form, undergoing ecdysis. *C*.—Advanced thoracic form, seventh day. *D*.—Slightly later thoracic form. *E*.—Proboscis form, ninth day. *F*.—Lateral and ventral views of caudal extremity of proboscis form.

The larvae taken into the gut of *Simulium* assume an activity which they do not possess when liberated from pieces of skin in warm water or saline solution. On the second day after ingestion forms can be found in the posterior thoracic muscles resembling in shape that seen in Fig. 1, A. They show anteriorly one or two small vacuolic areas interpreted as the excretory vesicle, and posteriorly a large vacuole opening to the exterior and often exuding a drop of clear fluid, the anus. The cells of the body in this stage are arranged more or less regularly in two sets longitudinally, a parietal and a central set, an arrangement which often gives the impression of there being a rudimentary alimentary canal, more particularly in the anterior part of the larva. These early thoracic forms are striking in shape and possess a very characteristic caudal appendage in the form of a spine which is, as a rule, straight but may be markedly curved. There is a great increase in size of these forms which seems to be accomplished, in part at least, by means of a process of ecdysis as shown in Fig. 1, B. At the conclusion of this stage there arises a more elongated larva which in place of the caudal spine has only small terminal papillae. This advanced form in the thorax occurs about the seventh day; its increase in length is accompanied by an increase in breadth; in it the alimentary canal becomes differentiated into several portions. At first it appears as in Fig. 1, C, having an anterior portion of the gut with large refractile cells and a mid-portion with the walls thin and the lumen wide and filled with fine yellowish granules, followed by a short wide flask-shaped portion with the anal opening at the surface. At this stage these portions of the gut are shut off, the one from the other, by apparently impervious constricted areas at the end of the anterior and mid portions. A more advanced stage is shown in Fig. 1, D, where the portions of the gut communicate with each other but yet in which considerable differences still exist between the portions. In Fig. 1, E, which represents a proboscis form of the ninth day, the worm is long and slender, the longest form attaining the length of over  $760\mu$  and a width of  $25\mu$  to  $18\mu$ , the commonest width being  $20\mu$ ; this stage possesses a patent alimentary canal of uniform lumen which runs straight from the anterior end to a narrow slit-like anus in front of the tail. In a few individuals in addition to the fine transverse striations visible under high powers

of the microscope there were seen elevated cuticular lines separated by several striae. The nerve ring is a fairly conspicuous structure in the advanced thoracic and head forms ; in some cases a short chain of cells was seen extending backwards from the nerve ring and situated between the parietal and the gut lines of cells.

Papillae could be made out in the anal region where one was seen to be situated on each side of the anus ; these were conspicuous. Two others, of much smaller size, were distinguished at the caudal extremity and in some cases a papilla situated laterally between the anus and the caudal extremity appeared to be present. The portion of the body between the anus appears to have a groove on the ventral surface, Fig. 1, F (lateral and ventral views), the margins of the groove ending in the proximity of, or actually in, the small papillae at the caudal extremity.

#### SUMMARY AND CONCLUSIONS

1. Larvae of *O. volvulus* taken up from the skin by *S. damnosum* in biting undergo progressive development in the fly and finally reach the proboscis ; the time taken to complete the development depends largely upon temperature.

2. The shortest period which elapsed after feeding before the proboscis became infected was seven days.

3. The mature larvae are found in the labium of the fly and escape through the membranous portion of it.

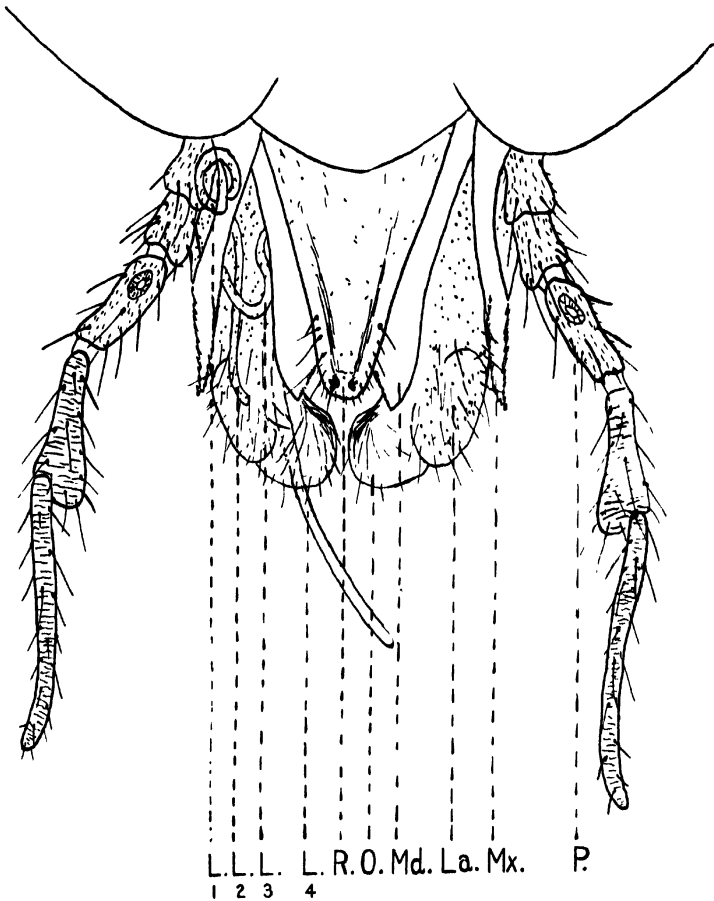
4. In so far as experiments with wild flies can be accepted as evidence in the absence of actual transmission to man or animal, *Simulium damnosum* is a vector of *Onchocerca volvulus*.



## EXPLANATION OF PLATE XIX

Mouth parts of *S. damnosum* fixed in alcohol cleared in warm clove oil and mounted; showing the position of the larvae of *O. volvulus*, emerging and *in situ*; semi-diagrammatic.

- L.* 1-4. Larvae of *O. volvulus*.
- R.* Labrum-epipharynx.
- O.* Labellum of Labium.
- Md.* Mandible.
- La.* Labium.
- Mx.* Maxilla.
- P.* Maxillary palp.





# MISCELLANEA

## *FASCIOLA HEPATICA* IN THE WILD RABBIT IN ENGLAND

On January 14th and 17th, 1925, Mr. J. Holroyd, F.R.C.V.S., of Blackburn, Lancs., sent me three rabbits, in the livers of which he had found flukes. The stomachs and intestines had been removed, so that only an inch or so of the rectum remained in each animal. The livers were greatly enlarged and contained 38, 33, and 22 adult flukes respectively. On being cleared, specimens of these were found to be morphologically similar to *Fasciola hepatica*. Coccidia and strongyle eggs were found in the faeces of each rabbit, and one had a small pisiform cysticercus in the abdominal cavity. Stock-owners often blame the rabbit for the spread of coccidiosis, tapeworm infestations, and strongylosis, especially in goats and sheep. The parasites of the rodent are, however, quite distinct from those of the ruminant, although it is a fact that both classes of animal are often seriously affected at the same time. Undoubtedly in such cases the climatic and other conditions have been favourable for the multiplication of parasites generally.

Although it is well known that the wild rabbit may harbour liver flukes, it is surprising how rarely one encounters them. A sportsman will forward rabbits which his keeper says are dying of fluke, but the autopsy will reveal a complete absence of them. Again, a zoological student may report 'all the rabbits with flukes in them,' and yet careful examination will reveal none. During the extensive outbreaks of liver fluke disease in sheep in recent years, one has rarely seen any reference to the rabbits in the same district, but it may be remarked that the rabbit is as a rule a lover of dry situations. The present cases are, therefore, not without interest, and curiously enough sheep have not had access to the land for over seven years, neither had the disease been recognised chronically in any of the farm animals in the surrounding district. There were appreciable losses in the rabbits early in 1924, and these increased considerably in January of 1925. Affected animals appeared to be confined to a particular belt of land, while on either side the rabbits seemed quite unaffected.

A. NOEL PILLERS.

28.12.25.



## A NOTE ON *COENURUS SERIALIS*

On November 24th, 1925, an uninfected dog was fed with a number of sub-cutaneous cysts (*Coenurus serialis*) from a rabbit.

Eggs were first found in the faeces on January 24th, 1926. The dog was treated on January 27th and 143 fully developed worms were recovered. A notable feature was that in many instances the worms were sterile, no trace of a uterus being present.

T. SOUTHWELL.

## AN OBSERVATION ON THE HATCHING OF A CESTODE EGG

It is sometimes stated that the liberation of a worm embryo, or larva, is affected by solution of the egg shell in the digestive juices of the host. Possibly this happens where the egg is provided with some special capsule or plug, and the shell is not homogeneous. The general rule, however, in Nematodes seems to be for the larva to rupture the egg-shell from within, and this may readily be seen to take place in Strongyloid eggs where the larva appears to strain forcibly against the shell.

In the case of Cestode eggs, recently, while watching the active boring movements of the hexacanth embryos in some eggs from a fresh, ripe segment of *Davainea cesticillus*, I observed one particularly vigorous embryo to tear its way first through the thin inner membrane, and afterwards through the shell of the egg, and so free itself altogether. Possibly this is what generally happens in cestode eggs, except where the embryo is enclosed in a specially developed embryophore.

E. L. TAYLOR.

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**Volume XX**

**August 13, 1926**

**No. 3**

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1925	Shah, Khwaja Samad
1925	Skan, Douglas A.
1925	Stone, Ernest R.
1925	Terrell, C. G.
1925	Tooth, Frederick
1925	de Waal, Jacobus Johannes
1926	Aitken, W. J.
1926	Ashworth, A.
1926	Bansikar, R. N.
1926	Bligh-Peacock, N.
1926	Bolton, Effie G.
1926	Boodrie, E. H.
1926	Brito-Mutunayagam, M. A. B.

*Date of  
Diploma*

1926	Cullen, T.
1926	Davies, H. E.
1926	Dias, B. G. V.
1926	Don, E. G.
1926	Fowler, H. P.
1926	Fowler, Isabella J.
1926	Hodgkinson, Katharine M.
1926	Jackson, R.
1926	Kamakaka, K. H.
1926	Lennox, D.
1926	Lewis, A. J.
1926	Mackay, A. G.
1926	McLean, N.
1926	MacSweeney, M.
1926	Malik, S. B.
1926	Merchant, M. E.
1926	Molony, E. F.
1926	Nashikkar, S. G.
1926	Oppenheimer, F.
1926	Paterson, F. S.
1926	Quigley, L. D.
1926	Rodrigues, N.
1926	Sachdev, A. S.
1926	Singh, B.
1926	Singh, J.
1926	Talib, S. A.
1926	Tan, C. L.
1926	Taylor, Catherine F.
1926	Turnbull, N. S.
1926	Vardya, B. K.
1926	Varma, T. N.

The following have obtained the Diploma in Tropical Hygiene of the University of Liverpool :—

*Diploma in Tropical Hygiene*

*Date of  
Diploma*

1926	Aitken, W. J.
1926	Bligh-Peacock, N.
1926	Clark, G.
1926	Collier, Ivy
1926	Cullen, T.
1926	Davis, B. L.
1926	Don, E. G. A.
1926	Fowler, H. P.
1926	Hawe, A. J.

*Date of  
Diploma*

1926	Lennox, D.
1926	Mackay, A. G.
1926	Mackay, D. M.
1926	McLean, N.
1926	MacSweeney, M.
1926	Oppenheimer, F.
1926	Skan, D. A.
1926	Talib, S. A.
1926	Turnbull, N. S.

# ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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Articles for publication should not exceed twenty-five pages of the *Annals*, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to:—The Editors, School of Tropical Medicine, The University, Liverpool.

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Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—‘According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.’ The references should be collected in alphabetical order of authors’ surnames at the end of the paper, and arranged in the following way:—

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SMITH, J. (1900). ‘Enlargement of the spleen in malaria.’ *Journal of Pathometry*, Vol. I, pp. 1-20.

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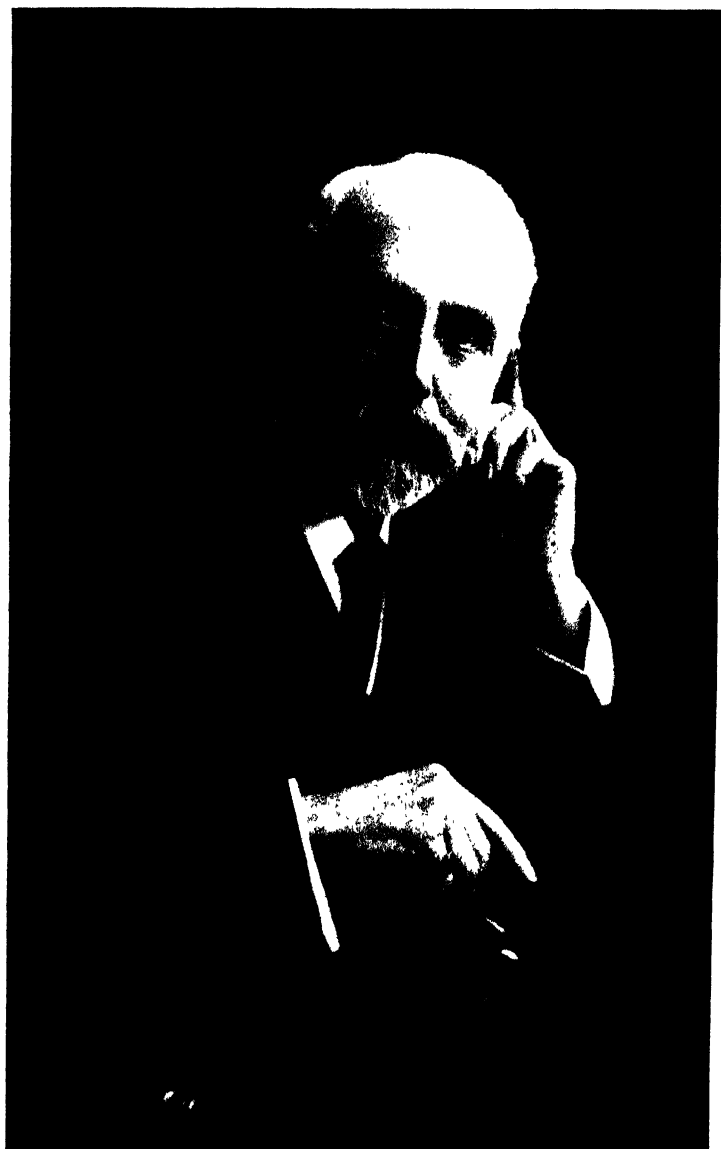


## SIR FRANCIS DANSON

By the death, on July 3rd, 1926, of SIR FRANCIS CHATILLON DANSON, the Liverpool School of Tropical Medicine has suffered a great loss. For many years Sir Francis had taken an active interest in, and had fostered in every way, the many activities of the School. He represented the Council of University College on the Committee from 1901 to 1902. From 1902 onwards he was a member of the Committee, and acted as Vice-Chairman from 1908 to 1913. In 1913, on the resignation of Lord Leverhulme, he accepted the unanimous invitation to become Chairman of the School, to which he has rendered many eminent services.

Under his leadership the work of the School was carried on with continued vigour, and important developments in various directions, which he was able to procure, added to the debt of gratitude which the School owes him. During his Chairmanship the School was transferred from its old laboratory in the University to its present building in Pembroke Place. During this period also, the African laboratory of the School, under the title of the Sir Alfred Lewis Jones Research Laboratory, was opened at Freetown, and a new Chair at the University established, namely, the Chair of Tropical Diseases of Africa, to be held by the Director of the Laboratory. Many important discoveries by the first Director, Professor D. B. Blacklock, and his Staff at the Sierra Leone Laboratory have resulted.

Sir Francis combined a kindly and charming personality with great foresight and breadth of vision, and all who came in contact with him will mourn the loss and cherish the memory of a good friend.



SIR FRANCIS C. DANSON



# CESTODES IN THE COLLECTION OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY  
T. SOUTHWELL

(Received for publication 26 April, 1926)

Sub-order **Univitellata** Southwell, 1925.

Family HYMENOLEPIDIDAE Railliet and Henry, 1909.

Sub-family (a) DIPYLIDIINAE Stiles, 1896.

Genus *Pancerina* Fuhrmann, 1899.

*Pancerina varanii* (Stossich, 1895) Sonsino, 1895.

Four specimens of this worm from *Varanus griseus*, Palestine, December, 1925, collected and presented by Dr. S. Adler.

The anatomy of the worm is illustrated in figs. 1, 2 and 3.

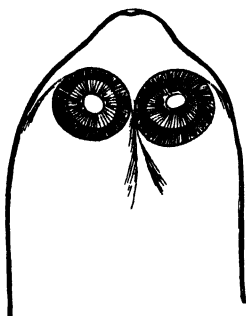


FIG. 1. *Pancerina varanii*. Head.  $\times 56$ .

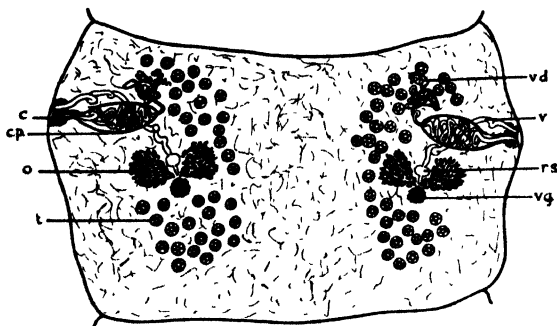


FIG. 2. *Pancerina varanii*. Mature segment. *c.*—cirrus; *c.p.*—cirrus pouch; *v.d.*—vas deferens; *t.*—testes; *v.*—vagina; *o.*—ovary; *r.s.*—receptaculum seminis, *v.g.*—vitelline glands.  $\times 56$ .

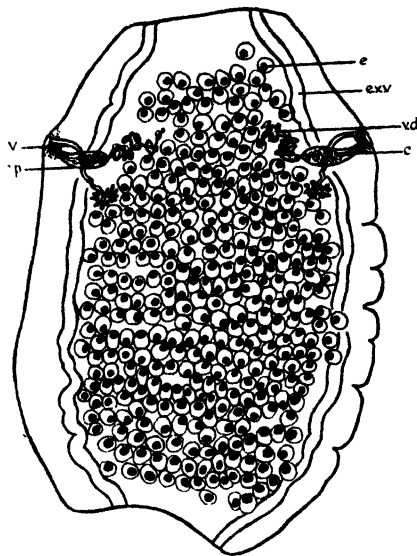


FIG. 3. *Poncirina taranis*. Gravid segment. *c*.—cirrus; *cp*.—cirrus pouch; *vd*.—vas deferens; *v*.—vagina; *e*.—eggs; *exv*.—excretory vessel.  $\times 35$ .

Sub-family (b) HYMENOLEPIDINAE Ransom, 1909.

Genus *Hymenolepis* Weinland, 1858.

*Hymenolepis lloydi* n.sp. Figs. 4, 5 and 6.

Four fragmented worms, with scolices, from the intestine of 'a large stork.' Azare, N.P. Nigeria, 21.x.25. Collected and presented by Dr. I.I. Lloyd.

**External anatomy.** The exact length of the worms could not be determined, but they are small and probably measure from 1 cm. to 2 cm. in length; the greatest breadth is 0.2 mm. The segments are numerous and they are all much broader than long; the genital pores are unilateral and are situated near the anterior margin of the segment.

**Head.** The head is globular and prominent; it measures 0.6 mm. in length and 0.35 mm. in breadth. The rostellum, when protruded, measures 0.18 mm. in length and 0.16 mm. in breadth. It is armed with twenty sickle-shaped hooks which vary in size from  $110\mu$  to  $140\mu$ . There is no neck.

**Internal anatomy.** Details of the muscular, nervous and excretory systems were not investigated, but it was noted that the muscular system was feebly developed.

**Male genitalia.** There are three large globular testes almost in a row ; in Mayhew's classification (1925) the worm falls in the genus *Hymenolepis*. The cirrus pouch is very large and prominent and extends almost half-way across the segment. Inside the pouch the

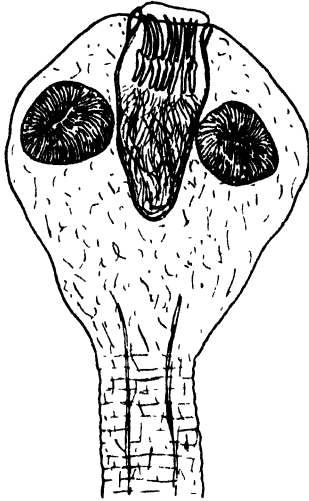


FIG. 4. *Hymenolepis lloydi*, n. sp. Head.  $\times 75$ .



FIG. 5. *Hymenolepis lloydi*, n. sp. Hooks.  $\times 330$ .

vas deferens is somewhat coiled and terminally it dilates into a small seminal vesicle. Outside the pouch the vas deferens is very long and

coiled and extends to the distal excretory vessel. No external seminal vesicle was seen.

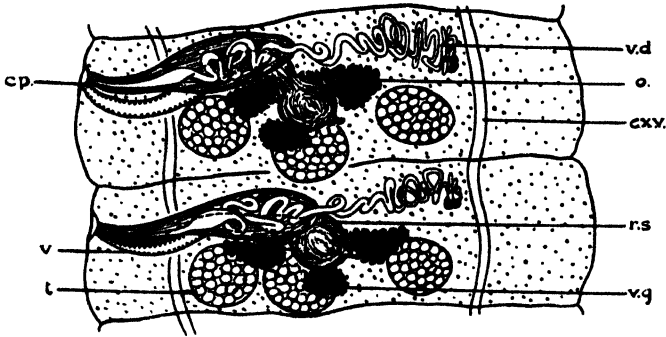


FIG. 6. *Hymenolepis lloydi*, n.sp. Mature segment. c.p.—cirrus pouch; v.d.—vas deferens; t.—testes; v.—vagina; o.—ovary; v.g.—vitelline glands; r.s.—receptaculum seminis; ex.v.—excretory vessel.  $\times 210$ .

*Female genitalia.* The ovary develops rather late and is a bilobed organ. From the pore the vagina dilates into a wide tube running ventral to the cirrus pouch; between the ovarian lobes it again dilates into a very large muscular receptaculum seminis. The vitelline gland is conspicuous and is situated posterior to the ovary.

*Uterus.* This organ was not fully developed in any of the available segments. It consisted of a narrow transverse tube situated in front of the ovary. No eggs were seen.

*Diagnosis.* This species differs from all known species of the genus *Hymenolepis* in the number and size of the hooks.

The writer has pleasure in naming the worm in honour of Dr. LL. Lloyd, to whom the School of Tropical Medicine is indebted for various collections of parasites obtained in Nigeria.

The type specimens, stained and mounted, are in the collections of the Liverpool School of Tropical Medicine.

Sub-order **Multivitellata** Southwell, 1925.

Family PROTEOCEPHALIDAE La Rue, 1914.

? *Proteocephalus pentastomum* Klaptocz, 1906.

One mature but non-gravid specimen of what appears to be this species from the small intestine of a siluroid mud-fish. Azare,

N.P. Nigeria, 11.xi.25. Collected and presented by Dr. I.I. Lloyd. The worm was strongly contracted and on this account it was impossible to make a definite diagnosis.

*Proteocephalus gallardi* Johnstone, 1911.

Numerous specimens presented by Dr. I.I. Lloyd from the intestines and inside pericardium of frogs. Azare, N.P. Nigeria. Collected on the following dates : 7 August, 1925, and 28 December, 1925.

It was at first thought that the worms were specimens of *P. hylae* (Johnstone, 1912), but a careful examination showed that they differed from that species (1) in size, (2) in the head being armed with innumerable spinules, and (3) in the cirrus being sometimes anterior and sometimes posterior to the vagina, whereas in *P. hylae* 'the male pore lies postero-dorsally to the female aperture, both terminating in a very short genital cloaca.'

Johnstone obtained *P. gallardi* from a black snake (*Pseudechis porphyriacus*) in Australia. It is now recorded from an African frog.

The writer was struck with the improbability of the same species occurring in both a snake and a frog, and in such widely separated areas, but the anatomy of the worm leaves no room for doubt.

The specimens measured over 40 cm. in length, and the head bore a terminal depression (apical organ).

Of doubtful systematic position :—

Genus *Diploposthe* Jacobi, 1896.

*Diploposthe laevis* (Bloch, 1782) Jacobi, 1896. Figs. 7 to 10.

One specimen from a 'small heron (*Ardea* sp.).' Azare, N.P. Nigeria, 20.viii.25. Collected and presented by Dr. I.I. Lloyd.

The specimen was peculiar in that in the anterior half of the strobila only a single set of male genital organs were present and these were unilateral. About the middle of the length of the worm the second set of male genitalia appeared suddenly, but irregularly. Posteriorly a double set of male genitalia was present in all segments except in four which bore a single set.

The female genital organs appeared about the middle of the



length of the worm: they were normal, except that in a few of the most posterior segments portions of the ovary had not atrophied and they were a conspicuous feature of segments in which otherwise only the cirrus pouches and uterus were visible.

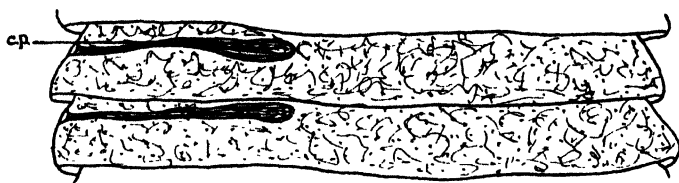


FIG. 7. *Diploposthe laevis*. Immature segment showing single male genitalia. *c.p.*—cirrus pouch.  $\times 75$ .

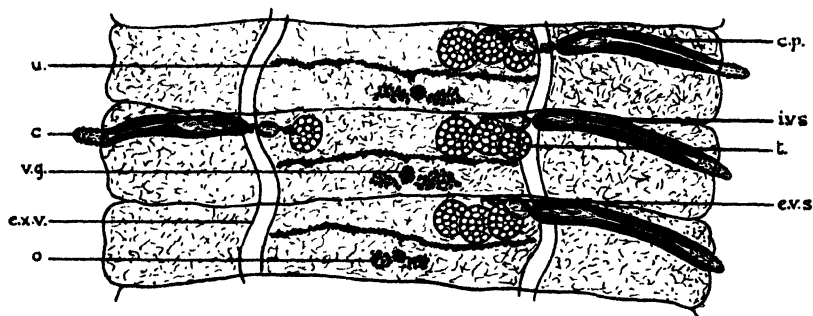


FIG. 8. *Diploposthe laevis*. Mature segment showing single and double genitalia. *c.p.*—cirrus pouch; *c.*—cirrus; *i.v.s.*—internal vesicula seminalis; *e.v.s.*—external vesicula seminalis; <sup>1</sup> *t.*—testes; *o*—ovary; *v.g.*—vitelline glands; *u.*—uterus; *ex.v.*—excretory vessel.  $\times 35$ .

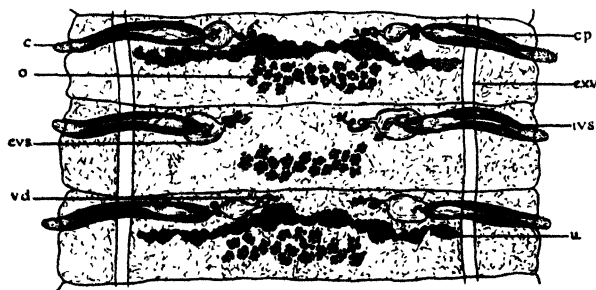


FIG. 9. *Diploposthe laevis*. Mature segment. *c.*—cirrus; *c.p.*—cirrus pouch; *i.v.s.*—internal vesicula seminalis; *e.v.s.*—external vesicula seminalis; *v.d.*—vas deferens; *o.*—ovary; *u.*—uterus; *ex.v.*—excretory vessel.  $\times 28$ .

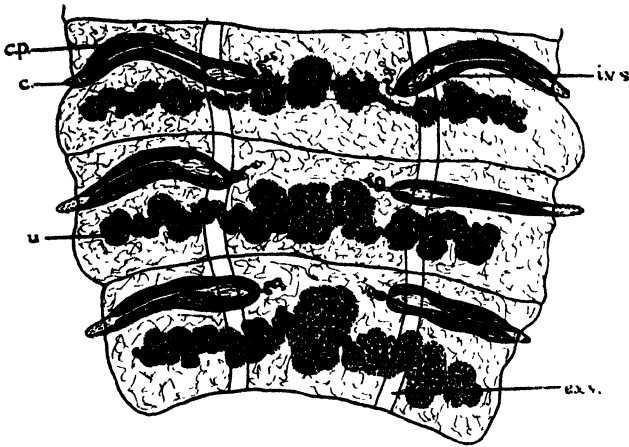


FIG. 10. *Diplostibe lacus*. Nearly gravid segment. *c*, cirrus; *cp*, cirrus pouch, *i.v.s.*—internal vesicula seminalis; *u*,—uterus; *ex.v.*—excretory vessel 35.

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# EMETINE PERIODIDE IN THE TREATMENT OF *S. HAEMATOBIIUM* INFECTIONS AMONGST WEST AFRICAN CHILDREN

BY

R. M. GORDON

(From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone)

(Received for publication 26 April, 1926)

The value of emetine in the treatment of schistosomiasis appears to have been first described by Hutcheson (1913), who recorded five cases of *S. japonicum* infection which improved markedly when treated with this drug. Curiously enough, no notice appears to have been taken of this important discovery until Diamantis (1917) recorded thirty cases of Egyptian bilharziasis successfully treated with emetine. The usual method of administering emetine in the treatment of schistosomiasis is to give from half to one and one-quarter grains of the hydrochloride subcutaneously, intramuscularly, or intravenously, till all symptoms have disappeared and live eggs are no longer passed in the urine; this treatment is then continued for a further variable period, the length of which depends upon the opinion of the individual physician as to what constitutes the minimum amount of emetine necessary to prevent a relapse. Emetine given in this manner appears to have no special advantages over antimony, except in the case of young children, the smallness of whose veins renders the intravenous injection of antimony difficult. Prolonged courses of either drug can readily be given to hospital patients, either at home or in the tropics. It is when the treatment of natives, and more especially the mass treatment of natives, in rural districts is attempted that the great drawback to these drugs becomes evident; both drugs require prolonged courses of injections and the native has a firmly rooted objection to this method of administering medicine, and will not tolerate a long

series of subcutaneous, intramuscular, or intravenous treatments ; even if this dislike be sufficiently overcome to allow of his receiving enough of the drug to relieve his more distressing symptoms, he will at this stage, almost inevitably, depart with his disease uncured and his potential danger to the community unlesened. That this is a very real difficulty is evident from the writings of numerous observers. To quote two instances : Blacklock (1925), writing of mass treatment by injections of antimony, states—‘ My personal opinion is that, situated as we are in Sierra Leone, it would be a waste of time and money to undertake it. I have not yet met a case there who would willingly continue to the end of a long course of treatment. The majority of cases relinquished treatment after about six injections ; if the patient declined to take more and was pressed to do so, it simply meant that urgent affairs quickly called him to a distant village. A mass retreat into the bush would be the chief result of mass treatment here.’ The figures for anti-bilharziasis work in Egypt, as quoted by Khalil (1924), would appear to show that of some fifty thousand cases treated less than half completed the prescribed course of twenty-two and a half grains of antimony ; the first figures regarding attendances given in his report are as follows :—

Rejected as unsuitable for treatment	...	...	...	...	...	...	...	63
Patients refused treatment	...	...	...	...	...	...	...	4
Patients treated with colloidal antimony	...	...	...	...	...	...	...	4
Patients receiving less than 5 grains of tartar emetic	...	...	...	...	...	...	...	313
"          "      5-10	"	"	...	...	...	...	...	215
"          "      10-15	"	"	...	...	...	...	...	146
"          "      15-20	"	"	...	...	...	...	...	92
"          "      20-25	"	"	...	...	...	...	...	55
"          "      25-30	"	"	...	...	...	...	...	84
"          over      30	"	"	...	...	...	...	...	19
Total								1,000

The later figures in this report corroborate the difficulty of persuading natives to complete their courses of treatment.

The West African native, although objecting strongly to all forms of injection, does not appear to extend this prejudice to medicines taken by the mouth and will travel great distances and report daily for long periods in order to obtain drugs of this description. It appears clear, therefore, that any drug capable of curing schistosomiasis by oral administration will prove of very great

value in the tropics, both for the treatment of individual cases, and still more so for the mass treatment of intensely infected areas where the drug can be distributed by a native dispenser, without incurring the risks associated with the giving of subcutaneous injections by an unqualified person. Leiper (1925), commenting on Blacklock's paper, remarks :—' It is on the question of the control of the disease, however, that I especially wished to speak, and I would put in a word for antimony as a prophylactic ; not as it is available at present, but through a simple method of giving antimony by the mouth, which, I hope, further work may reveal. Measures which will involve a combination of antimony by the mouth, with some method of attacking the snail, are those upon which I imagine ultimate success will depend.'

So far as the present writer is aware, no preparation of antimony suitable for the treatment of schistosomiasis by oral administration has as yet appeared on the market ; but the figures which follow appear to suggest that in emetine periodide we have a drug which may prove of great value in the oral treatment of schistosomiasis in the tropics.

#### EMETINE PERIODIDE.

Emetine periodide was first prepared and described by Martindale (1923). Willmore (1923) treated ninety-one cases of amoebic dysentery with emetine periodide and recorded forty-three (47 per cent.) as presumably cured and forty-eight (53 per cent.) as having relapsed. Gordon (1923) treated sixteen cases of amoebic dysentery with the same drug, ten cases (62 per cent.) relapsed. Willmore refers to emetine periodide in the following terms :—' So far, emetine periodide appears to be by far *the most effective, and, at the same time, the least toxic, of all the emetine preparations which I have tried.* . . . . E.P.I. (as emetine periodide may be called for short) has the great advantage of allowing an intensive course of 90 grains to be given in the short period of fifteen days, and, if necessary, this may be repeated after about ten days' interval ; compared with E.B.I., it therefore materially shortens the period of hospitalisation, and thereby affects a distinct economy in hospitals and pensions administration, in spite of its high price.'

## EXAMINATION AND CLASSIFICATION OF CASES

An examination of eighty-one children at the 'United Brethren in Christ' Missionary School, Jiamā, Nimmi Korro, showed that forty-three of them were suffering from *S. haematobium* infection of the bladder; of these forty-three cases, twenty-eight were severe cases; that is to say, they were passing many live eggs and much blood in every sample of urine examined. These twenty-eight severe cases were divided into two classes of fourteen each, care being taken that the average degree of severity of infection was about equal in the two classes; one of these classes (Class I) received emetine hydrochloride treatment given subcutaneously, the other (Class II) emetine periodide treatment given by the mouth. The ages of the twenty-eight cases varied from six to seventeen years, the average age of the emetine hydrochloride group being eleven years and six months, and that of the emetine periodide group nine years and seven months; the age of each child is given in the tables and it can be seen that the result of the treatment seems to be quite uninfluenced by the age of the child.

The urine of each of the twenty-eight cases was examined microscopically every alternate day during the treatment and for eight days after the completion of the course. During the first three days of treatment, while all the children were still passing large numbers of live eggs and no dead eggs, the deposit from an uncentrifuged specimen of urine was examined; from the third day onwards all urines were centrifuged before being examined. In cases where the actual number of eggs per cover-slip preparation is not shown the sign +++ is used to denote a heavy infection, ++ to denote a moderate infection, and + to denote a scanty infection.

### CLASS I. SUBCUTANEOUS INJECTIONS OF EMETINE HYDROCHLORIDE

In Table I is shown the curative effect of emetine hydrochloride treatment on fourteen West African children aged nine to seventeen, who were suffering from severe infections of *S. haematobium*. Cases 1-13 received half a grain of emetine hydrochloride, given subcutaneously, once a day on fifteen consecutive days. Case 14 missed four days' treatment, but only one examination, about the

TABLE I.

Showing the curative effect of subcutaneous injections of emetine hydrochloride on fourteen West African children severely infected with *S. baematothium*.

		Before Treat- ment	Days after first day of treatment										
			1	3	5	7	9	11	13	15	17	19	21
Case 1 Male Age 9	Live eggs ...	+++	++	+++	++	+	o	o	o	o	o	o	o
	Dead eggs	o	o	o	o	+	++	o	2	o	o	o	o
Case 2 Male Age 9	Live eggs ...	+++	++	+	+	o	o	2	2	o	o	o	o
	Dead eggs	o	o	o	+	1	o	1	o	1	o	o	o
Case 3 Male Age 12	Live eggs	+++	+++	+++	+++	+++	++	+	+	+	+	o	o
	Dead eggs	o	o	o	o	o	++	++	+	++	++	8	4
Case 4 Male Age 11	Live eggs ...	+++	+++	+++	++	++	1	o	1	1	2	o	o
	Dead eggs	o	o	o	++	o	1	+	o	o	o	3	++
Case 5 Male Age 10	Live eggs ...	+++	++	++	+	+	o	+	o	o	o	o	o
	Dead eggs	o	o	o	o	+	+	+	1	1	o	o	1
Case 6 Male Age 14	Live eggs ...	+++	+++	++	++	++	o	o	o	2	1	o	o
	Dead eggs	o	o	o	o	o	+	o	o	o	2	o	o
Case 7 Male Age 16	Live eggs ...	+++	+++	+	o	o	1	o	o	o	o	o	o
	Dead eggs	o	o	o	+	o	o	o	o	o	o	o	o
Case 8 Male Age 9	Live eggs ...	+++	+++	+++	++	+	o	1	o	o	o	o	o
	Dead eggs	o	o	o	++	o	+	o	+	o	o	o	o
Case 9 Male Age 10	Live eggs ...	+++	+++	+++	++	++	+	o	2	o	2	o	o
	Dead eggs	o	o	o	o	++	+	o	o	1	10	1	1
Case 10 Male Age 10	Live eggs ...	+++	+++	+++	++	++	o	o	o	o	o	o	o
	Dead eggs	o	o	o	o	++	1	1	o	o	1	o	o
Case 11 Male Age 9	Live eggs ...	+++	+++	+++	+++	++	++	o	o	o	o	o	o
	Dead eggs	o	o	o	o	o	+	o	1	o	1	3	o
Case 12 Female Age 12	Live eggs ...	+++	+++	+++	+++	+++	++	++	++	++	1	5	o
	Dead eggs	o	o	o	o	o	++	++	+	++	o	5	2
Case 13 Female Age 13	Live eggs ...	+++	+++	+++	+	+	o	o	+	o	o	o	o
	Dead eggs	o	o	o	o	+	+	o	++	o	o	o	1
Case 14 Male Age 17	Live eggs ...	+++	++	+	++		o	o	o	o	o	o	o
	Dead eggs	o	o	o	o		1	o	1	o	o	o	o



middle of the course, and was given four days' extra treatment at the end of the course. Considerable difficulty was experienced in making the children attend for their injections and disciplinary action had to be taken to ensure that each child completed its full course. Several cases of 'emetine nodules' and sore arms resulted from these fifteen days of consecutive injections, while one child, four days after the completion of his treatment, developed some rather alarming signs of heart failure which disappeared after two days' rest in bed.

## CLASS II. ORAL ADMINISTRATION OF EMETINE PERIODIDE

In Table II is shown the curative effect of emetine periodide, given by the mouth, on fourteen West African children, aged six to thirteen years, who were suffering from severe infections of *S. haematobium*. Cases 1-10 received one grain of emetine periodide orally three times a day on fifteen consecutive days; cases 11, 12, and 13 received the same treatment but missed one day's treatment about the middle of the course and were given an extra day's treatment at the end of the course. Case 14 left school after completing ten consecutive days of treatment. The emetine periodide was supplied in gelatine capsules, but owing to previous experience of gelatine capsules passing through the gut unchanged the powder was removed from the capsule and given mixed with a little milk. It was previously noted by the writer (Gordon 1923) that this method of administering the drug never produced vomiting and the present series of cases confirmed this observation; amongst a total of six hundred and thirty doses administered, vomiting only occurred once, and as the same child completed its course without any further trouble the vomiting was probably independent of the drug. No unpleasant symptoms, such as those recorded with the use of emetine hydrochloride, followed the taking of emetine periodide by mouth, and it is important to note that no difficulty whatsoever was experienced in getting the children to report for, and take, their medicine.

It will be seen from Table I that all fourteen children treated with emetine hydrochloride subcutaneously had ceased to pass live ova at the end of the eight-day observation period. Table II

TABLE II.

Showing the curative effect of oral administration of emetine periodide on fourteen West African children severely infected with *S. baematobium*.

		Before Treat- ment	Days after first day of treatment										
			1	3	5	7	9	11	13	15	17	19	21
Case 1 Female Age 7	Live eggs ...	+++	+++	++	+	+	+	+	+	0	0	0	0
	Dead eggs	0	0	0	0	+	+	+	+	20	4	7	2
Case 2 Male Age 9	Live eggs ...	+++	+++	+++	+++	+++	++	++	++	++	++	++	++
	Dead eggs	0	0	0	0	0	0	++	+	+	0	+	+
Case 3 Female Age 12	Live eggs ...	+++	+++	+++	+++	++	+	+	++	++	+	+	0
	Dead eggs	0	0	0	0	0	+	++	+	+	++	++	++
Case 4 Male Age 10	Live eggs ...	+++	++	+++	++	+	0	2	0	0	0	0	0
	Dead eggs	0	0	0	0	0	+	0	0	1	0	0	0
Case 5 Male Age 6	Live eggs ...	+++	+++	++	+++	++	0	0	0	0	0	0	0
	Dead eggs	0	0	0	0	0	1	0	0	0	3	0	0
Case 6 Male Age 8	Live eggs ...	+++	+++	++	+++	+	0	0	5	0	1	0	0
	Dead eggs	0	0	0	0	+	2	4	2	10	0	1	0
Case 7 Male Age 10	Live eggs ...	+++	+++	++	+	+	1	0	0	0	+	+	0
	Dead eggs	0	0	0	0	+	0	2	2	5	+	+	1
Case 8 Male Age 8	Live eggs ...	+++	+++	+++	++	+	0	1	0	0	0	0	0
	Dead eggs	0	0	0	0	0	0	1	0	0	0	3	0
Case 9 Male Age 11	Live eggs ...	+++	+++	++	+++	0	+	+	+	0	0	0	1
	Dead eggs	0	0	0	0	0	0	+	+	2	++	4	+
Case 10 Male Age 11	Live eggs ...	+++	++	+++	++	0	0	0	0	7	2	1	0
	Dead eggs	0	0	0	0	2	+	2	0	11	8	3	0
Case 11 Male Age 8	Live eggs ...	+++	+++	++	++	+	0	0	0	0	0	0	0
	Dead eggs	0	0	0	0	0	0	0	0	1	0	0	0
Case 12 Male Age 8	Live eggs ...	+++	+++	++	+++	++	0	0	+	0	0	0	0
	Dead eggs	0	0	0	0	+	0	0	0	1	0	1	10
Case 13 Male Age 13	Live eggs ...	+++	+++	+++	++	++	+	+	+	2	0	0	0
	Dead eggs	0	0	0	++	++	+	+	+	8	3	1	++
Case 14 Male Age 13	Live eggs ...	+++	++	++	++	+	0						
	Dead eggs	0	0	0	++	++	1						

shows that twelve of the fourteen children, treated with emetine periodide by the mouth, had ceased to pass live ova at the end of the same observation period, while the remaining two cases were passing a mixture of live and dead eggs. The Tables also show that with both forms of treatment the urinary findings of the patients continued to improve for some time after the cessation of treatment, so that it is possible that if the observation period had been longer these two cases might also have become negative. It is, of course, impossible to predict how many of the apparently cured cases will relapse. It was at first proposed to re-examine the children a few months after the completion of their treatment, but as all the cases are living in an intensely infected area, a positive finding under such conditions would be of no value. The absence of all unpleasant symptoms following the oral administration of one grain of emetine periodide three times a day for fifteen consecutive days suggests that a larger dose might possibly have been employed with safety.

### CONCLUSIONS

1. The oral administration of emetine periodide clears up the urine of children, intensely infected with *S. haematobium*, just as quickly, and almost with as great certainty, as subcutaneous injections of emetine hydrochloride.
2. Certain ill-effects were noted as the result of subcutaneous injections of emetine hydrochloride, and great difficulty was experienced in making the children report for treatment. No ill-effects followed the oral administration of emetine periodide, and no difficulty whatsoever was experienced in getting the children to attend for, and take, this drug.

### ACKNOWLEDGMENTS

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# MALARIA IN THE CHILDREN OF FREETOWN, SIERRA LEONE

BY

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*(From the Sir Alfred Lewis Jones Research Laboratory, Freetown,  
Sierra Leone)*

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## INTRODUCTION

With a view to finding out the amount of malarial infection in the school children of Freetown, and its effect on the child's health, the following investigation was made between July, 1925, and March, 1926. This includes the greater part of the rainy season and four months of the dry season, the rainfall figures for this period, taken from the Sierra Leone Royal Gazette, being :—

July ... ..	30·72 inches
August ... ..	34·38 "
September ... ..	22·88 "
October ... ..	19·74 "
November ... ..	7·4 "
December ... ..	0·15 "
January ... ..	0·0 "
February ... ..	0·0 "
March ... ..	0·12 "

The work was carried out in conjunction with Dr. M. G. Blacklock, Lady Medical Officer, to all of whose figures I have had the advantage of ready access. Seven schools in different parts of the town were visited and the blood and spleen of every child and, in most cases, the temperature, was examined. The results of the examinations will first be described and an attempt then made to correlate them.

## I. EXAMINATION OF SPLEEN

### *(a) Description of method employed.*

For some time past an accurate method of measuring the spleen of children in a community undergoing a malaria survey has been needed. Christophers (1924a) and Christophers and Khazan Chand (1924) suggested a method of triangulation of the apex of the spleen and a method of correcting the measurement thus found for the size of the child ; their work has been followed in this enquiry, except that new standard measurements suitable for the African child have been made.

The position of the apex, or most projecting part of the spleen, is triangulated by measuring its distance from the mid-line and from the umbilicus. These measurements having been taken there is only one possible position for the apex above the umbilicus (if the apex is below the umbilicus this is noted at the time of examination).

These initial measurements cannot be used in a survey as the same measurement of, say, 10 cms. from the umbilicus would mean a very different sized spleen in a large child from that in a small child. For this reason the initial measurement has to be multiplied by a correction factor based on the size of the child. The following is a broad outline of the method employed in preparing this correction factor, the full details being given subsequently. A child of 60 cms. sitting height was taken as a standard and a standard abdominal chart prepared by examination of over 1,000 children, showing the measurements of that portion of the abdomen within which the spleen lies. In addition to this the ratio of the rate of increase of abdominal measurements to the rate of increase of sitting height was found. Once these two, the standard measurements and the ratio, were known, a formula was prepared by means of which any measurement of a child of known sitting height could be altered

so as to become comparable with one in a child of 60 cms. sitting height. In order to avoid the necessity for measuring the sitting height of every child the formula has been prepared in such a manner that, in practice, the inter-nipple or nipple-umbilical lines might be substituted for it.

*Preparation of an abdominal chart.* Christophers (1924a) prepared a chart suitable for Indian children; as some doubt was felt as to whether this was applicable to the African child a new one was prepared in a manner similar to his. The sitting height and a number of other measurements were made of each child, the average of each of these was found and, by means of a correlation table and Galton graph, showing the ratio of variation between these measurements and the sitting height, these averages were reduced to the value they would have in the standard child. The chart is drawn in the following manner:—

Draw a horizontal line 13·1 cms. long, representing a line drawn from the median line of the body to the mid-axillary line at the level of the nipples; at a point 6·3 cms. from the left end of this, mark a point representing the nipple; draw a line vertically downwards from each end of this horizontal line, the left representing the median line of the body, the right one the mid-axillary line; a line drawn from the nipple so as to cut the median line 19·5 cms. from the nipple, will represent the nipple-umbilical line. The costal margin is represented by a curved line cutting the median line 2·5 cms. from the inter-nipple line, the nipple-umbilicus line 7·6 cms. from the nipple, and the mid-axillary line 10·4 cms. from the nipple-axillary line. The chart thus formed, which is reproduced in fig. 1, represents the left upper quadrant of the abdomen and lower quadrant of the chest drawn in a single plane instead of being curved.

The rate of increase of abdominal measurements is taken to be the same as that of the nipple-umbilicus line. When the different values of this and the sitting height are compared by means of a correlation table and Galton graph, it is found to have a ratio of variation of 0·8; that is, for every increase of 1 per cent. in the sitting height there is an increase of 0·8 per cent. in the nipple-umbilicus line and in other abdominal measurements.

As our standard is a child of a certain sitting height it would be correct, in all cases where it is desired to use this correction



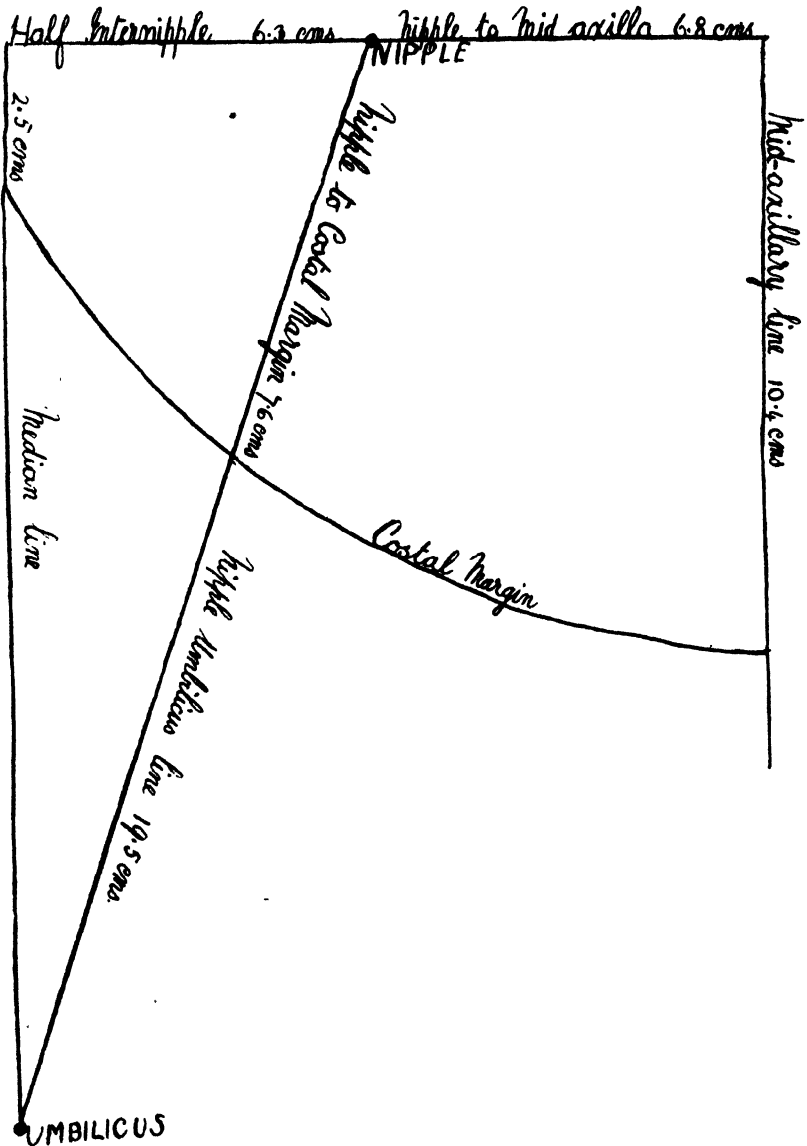


FIG. 1. Standard abdominal chart, drawn in the manner described on p. 241.

factor, to take the sitting height of the child as an indicator measurement; this, however, is an inconvenient measurement to take, whereas the nipple-umbilicus line and inter-nipple line are always easily measured at the time of triangulation of the spleen. Any one of these three may therefore be taken and the splenic measurement corrected by reference to Table I, in the vertical columns of which are the corrected values of the observed measurement for different values of the sitting height, inter-nipple line and nipple-umbilicus line.

The use of these two thoracic and abdominal measurements as indicators is justified by convenience when a number of children are being examined and the average size of spleen in a district is wanted. In the case of individual children, however, it is not justified and the use of anything but the sitting height as an indicator may lead to errors of 2 cms. in the corrected value of a measurement. When a number of children are being examined, however, these inaccuracies, which are only few and rarely as great as the above, are negligible, as may be seen by reference to Table II, in which the children are divided into small groups and the average distance of the spleen from the umbilicus is given corrected by each of the three indicator measurements. Children in whom the spleen was recorded as 'at the costal margin' or 'palpable' and children in whom not all three indicators were measured are not included in this table, so that the numbers are smaller than in succeeding ones.

### *(b) Application of method in practice.*

*Method of measurement of the spleen.* The spleen is palpated preferably with the child in the standing position---when necessary the child may be told to bend down and breathe deeply---and if palpable the position of the apex is marked on the abdominal wall with a grease pencil. The distance of this point from the mid-line and from the umbilicus, and the length of one of the indicator measurements are taken with a tape marked in centimetres; if the spleen is below the umbilicus or is 'at the costal margin' or only 'palpable but not reaching the costal margin' the fact is noted.

These data are entered in a book and at a later time the splenic measurements are corrected. If it is desired to obtain only the average distance of the apex from the umbilicus this is sufficient;

TABLE I.

Correction Table. The vertical columns show the corrected values of the measurements in the top horizontal column for different values of the sitting height (S.H.), inter-nipple line (I.N.), and nipple-umbilical line (N.U.).

S.H.	I.N.	N.U.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
40	...	14	1	3	4	5	7	8	10	11	12	14	15	16	18	19	20	22	23	25	26	27
41	9	...	1	3	4	5	7	8	9	11	12	13	15	16	17	19	20	21	23	24	25	27
42	...	...	1	3	4	5	7	8	9	11	12	13	14	16	17	18	20	21	22	24	25	26
43	...	15	1	3	4	5	6	8	9	10	12	13	14	16	17	18	19	21	22	23	25	26
44	...	...	1	3	4	5	6	8	9	10	11	13	14	15	17	18	19	20	22	23	24	25
45	...	...	1	2	4	5	6	7	9	10	11	12	14	15	16	17	19	20	21	22	24	25
46	10	...	1	2	4	5	6	7	9	10	11	12	14	15	16	17	18	20	21	22	23	25
47	...	16	1	2	4	5	6	7	8	10	11	12	13	15	16	17	18	19	21	22	23	24
48	...	...	1	2	4	5	6	7	8	10	11	12	13	14	15	17	18	19	20	21	23	24
49	...	...	1	2	4	5	6	7	8	9	11	12	13	14	15	16	18	19	20	21	22	23
50	...	17	1	2	3	5	6	7	8	9	10	12	13	14	15	16	17	18	20	21	22	23
51	...	...	1	2	3	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20	22	23
52	11	...	1	2	3	4	6	7	8	9	10	11	12	13	15	16	17	18	19	20	21	22
53	...	...	1	2	3	4	6	7	8	9	10	11	12	13	14	15	17	18	19	20	21	22
54	...	18	1	2	3	4	5	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22
55	...	...	1	2	3	4	5	6	8	9	10	11	12	13	14	15	16	17	18	19	20	21
56	...	...	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	19	20	21
57	12	...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
58	...	19	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21
59	...	...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
60	...	...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
61	...	...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
62	13	20	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	17	18	19
63	...	...	1	2	3	4	5	6	7	8	9	10	11	12	12	13	14	15	16	17	18	19
64	...	...	1	2	3	4	5	6	7	8	9	10	10	11	12	13	14	15	16	17	18	19
65	...	...	1	2	3	4	5	6	7	7	8	9	10	11	12	13	14	15	16	17	18	19
66	...	21	1	2	3	4	5	6	6	7	8	9	10	11	12	13	14	15	16	17	18	19
67	14	...	1	2	3	4	5	5	6	7	8	9	10	11	12	13	14	15	16	17	17	18
68	...	...	1	2	3	4	5	5	6	7	8	9	10	11	12	13	14	14	15	16	17	18
69	...	...	1	2	3	4	4	5	6	7	8	9	10	11	12	12	13	14	15	16	17	18
70	...	22	1	2	3	4	4	5	6	7	8	9	10	11	11	12	13	14	15	16	17	18
71	...	...	1	2	3	3	4	5	6	7	8	9	10	10	11	12	13	14	15	16	17	17
72	15	...	1	2	3	3	4	5	6	7	8	9	9	10	11	12	13	14	15	16	16	17
73	...	23	1	2	3	3	4	5	6	7	8	9	9	10	11	12	13	14	14	15	16	17
74	...	...	1	2	3	3	4	5	6	7	8	8	9	10	11	12	13	13	14	15	16	17
75	...	...	1	2	3	3	4	5	6	7	7	8	9	10	11	12	13	13	14	15	16	17
76	...	...	1	2	2	3	4	5	6	7	7	8	9	10	11	12	12	13	14	15	16	16
77	...	24	1	2	2	3	4	5	6	7	7	8	9	10	11	11	12	13	14	15	15	16
78	16	...	1	2	2	3	4	5	6	6	7	8	9	10	10	11	12	13	14	15	15	16
79	...	...	1	2	2	3	4	5	6	6	7	8	9	10	10	11	12	13	14	14	15	16
80	...	25	1	2	2	3	4	5	6	6	7	8	9	9	10	11	12	13	13	14	15	16

In the few cases where greater accuracy is required than can be got from the table, use may be made of the following formula which is a slight modification of that given by Christophers.

$$A = a \frac{1}{1 + \frac{r}{R} \left( \frac{h}{H} - 1 \right)}$$

Where  $A$  is the required abdominal measurement.

Where  $a$  is the observed abdominal measurement.

Where  $r$  is the ratio of variation of abdominal measurements (0.8).

Where  $R$  is the ratio of variation of indicator measurement taken.

Where  $b$  is the observed indicator measurement.

Where  $H$  is the standard indicator measurement.

The ratio of variation and 'standard lengths' are:—

Sitting height ...	...	...	...	...	...	1.0	60 cms.
Inter-nipple line ...	...	...	...	...	...	0.9	12.6 cms.
Nipple-umbilicus line...	...	...	...	...	...	0.8	9.5 cms.

Where the nipple umbilicus line is used as an indicator  $\frac{r}{R} = 1$  and when the sitting height is

used  $\frac{r}{R} = .8$ .

but if it is desired to study the path of descent of the spleen the corrected values can be entered on the chart. Christophers (1924a) gives the following directions for doing this 'since every double measurement indicates a definite point on the abdomen the position of the apices of the spleens in any series can be marked on the standard abdominal chart. For this purpose the chart may be ruled with two sets of lines, one being circles giving distances of 1, 2, 3, etc., cms. from the umbilicus, the other being straight lines drawn parallel to the median line of the body at distances of 1, 2, 3, etc., cms.

TABLE II.

Showing the average size of spleen in each school. Spleens projecting below the costal margin alone included.

School	Number measured	Average distance from umbilicus corrected by		
		Sitting height	Internipple line	Nipple-umbilicus line
Model School ... ..	22	7·8 cms.	7·9 cms.	7·8 cms.
Cathedral Infants .. ...	33	7·3 cms.	7·3 cms.	7·3 cms.
Holy Trinity ... ..	39	9·0 cms.	9·1 cms.	8·9 cms.
St. Edward's ... ..	22	9·9 cms.	9·8 cms.	9·7 cms.
Bethel Wesleyan ... ..	59	9·3 cms.	9·4 cms.	9·5 cms.
St. Joseph's ... ..	60	8·1 cms.	8·3 cms.	8·3 cms.
St. Anthony's ... ..	130	8·8 cms.	8·8 cms.	8·8 cms.
Buxton ... ..	80	8·7 cms.	8·6 cms.	8·8 cms.

So that the spaces and not the lines should correspond to these measurements, the standard chart has been ruled with lines at distances of  $1\frac{1}{2}$ ,  $2\frac{1}{2}$ ,  $3\frac{1}{2}$ , etc., cms. ; the spaces therefore correspond to measurements of 1, 2, 3, etc., cms. The lines form a series of diamond-shaped spaces each equivalent to a spleen measurement on the double notation. Into each space, therefore, can be entered the number of spleens showing this particular measurement.'

A chart constructed in this manner giving the position of the apices of the spleens of the hyper-endemic area, referred to later, is shown in fig. 2.

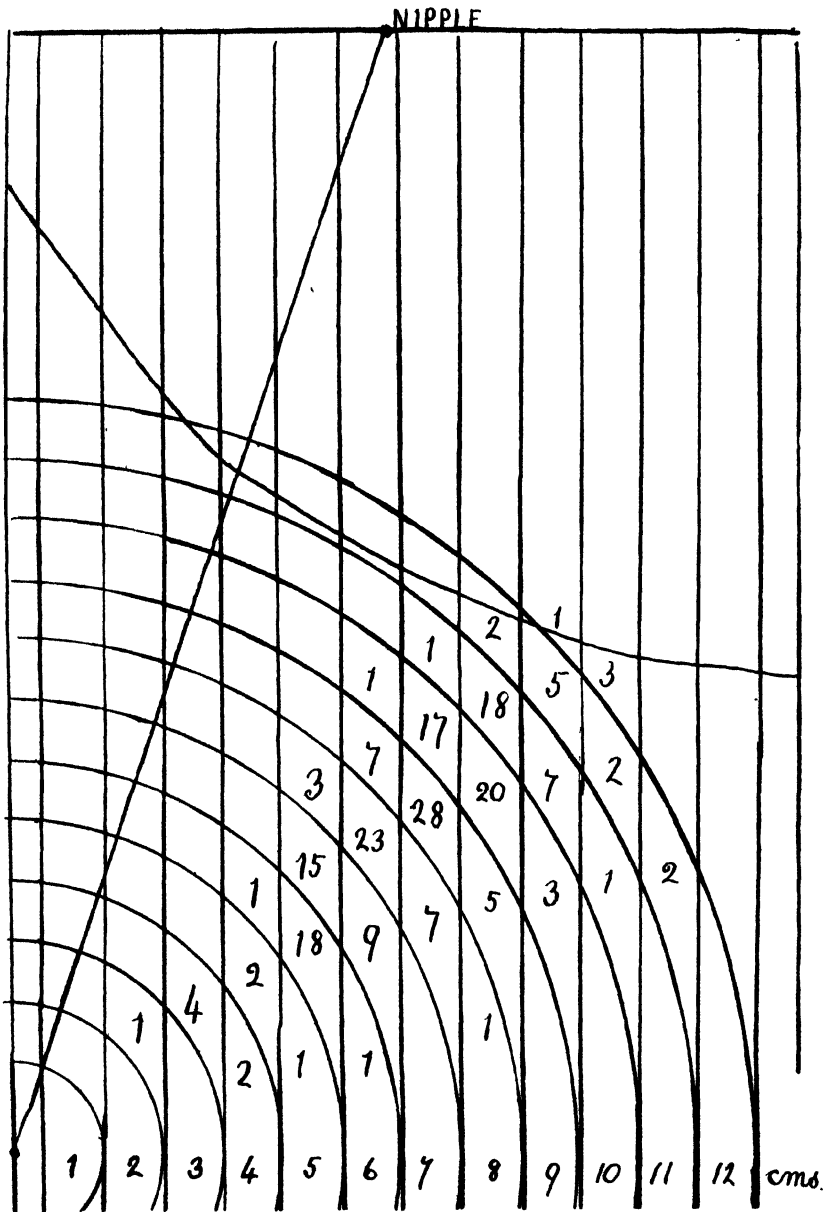


FIG. 2. Standard abdominal chart, ruled as suggested by Christophers, with the spleens of the hyper-endemic area entered on it. Owing to the limitations of space, spleens projecting below the umbilicus are not shown. The figures represent the number of apices in each space.

Christophers (1924a) states that for the expression of a value for the size of the enlarged spleen in a community the position of the mean on the progression line of the apex is required. This has a serious disadvantage, in that no value is given by this method to spleens which cannot be measured because they are only 'at the costal margin' or 'palpable but not reaching the costal margin.' He was, however, working in a hyper-endemic area where such spleens formed no large part of the whole, but in some districts in Freetown, districts where the severity of malarial infection is slight, such spleens may form half of the palpable spleens and their omission when calculating the mean would lead to serious error. This difficulty could be surmounted by assigning to these spleens their proper value. The malarial spleen usually passes under the costal margin in the standard child at a point about 13 cms. from the umbilicus; the normal position of the apex of the un-enlarged spleen is probably in the mid-axillary line about 17 cms. from the umbilicus in the standard child; and the 'just palpable' spleen probably lies, on the average, about half-way between the two, i.e., 15 cms. from the umbilicus. In calculating the mean distance of the spleen from the umbilicus the 'costal margin' and 'just palpable' spleens might be given respectively the values of 13 and 15 cms.; the mean would then give a much more complete picture of the degree of splenic enlargement.

### (c) *Results of Examination.*

One thousand and fifty-nine children were examined in the manner described, and of those 604 (57 per cent.) had palpable spleens. The number of children aged ten or under examined was 852; of these 475 had palpable spleens so that the spleen index of Ross (1910) is 56.

It became obvious later in the enquiry that part of the town, the extreme West, came under Christophers' (1924b) definition of a hyper-endemic area—one in which the spleen rate (children) is permanently over 50 per cent.—and part, the Central and Eastern portion, that of an endemic area—areas, i.e., showing moderate to high, but often variable, spleen rates not permanently over 50 per cent. This difference was noted despite the fact that the endemic

area was examined during what is normally the most malarious season of the year, the wet season and early part of the dry season, while the hyper-endemic area was examined during the latter part of the dry season, the relatively healthy period before the first showers of the year.

The Central and Eastern part of the town is, for the most part, on a slight but definite slope, and has two streams running through it—Nicol's Brook and the stream opening at Magazine Wharf. The extreme Western portion of the town is low-lying and through it run Sanders Brook and Alligator Brook.

Blacklock and Evans (1926) in discussing the distribution of *A. costalis*, the commonest Freetown anopheline, state that its larvae were found in all four of these streams, but whereas in Nicol's Brook they were mainly found below and on the outskirts of the town, and in the Magazine Wharf stream only below the town, in Sanders Brook it was notable that they occurred where the laterite drains from the streets join the stream, actually in the town. In the fact that the commonest vector is here breeding in the near vicinity of the house, even at the end of the dry season, we appear to have the explanation of the heavier infection in the Western part. As these two areas present marked differences they will here be described separately.

### (1) *Endemic Area.*

Of the total 1,059 examined, by far the majority, 722, of the children seen came from this district and of these 360 (50 per cent.) had enlarged spleens; the percentage of children of ten and under with enlarged spleens was 49, and the average distance of the spleen from the umbilicus, including palpable and costal margin spleens, was 10.5 cms. These spleens were not, however, distributed evenly along the line of progression, but were grouped about two distinct sizes, one a small size, consisting of spleens not projecting below the costal margin—123 spleens fell within the class—and a larger size, consisting of spleens 7-10 cms. from the umbilicus, into which group fell 150 spleens; the intervening medium size and the larger sizes only accounting for forty-eight and thirty-nine respectively. This is in agreement with the work of Christophers (1924b), who found that the spleens he examined in the Singbhum

district fell into three classes, small, medium, and large (though the spleens in each group were larger than those in the corresponding group here) ; the medium group was characteristic of the period of 'acute infestation' that is, the first two or three years of life, after which, in the period of 'immune infestation' ('premunition' (Sergeant, Parrot, and Donatien, 1925) ) the small and large groups grew at its expense. In the Freetown investigation practically all the children examined were well within the period of immune infestation, so that it is not unnatural that there should here be such a marked separation into two main groups.

*Relation between the spleen and parasite findings.* That there is a definite relation between the presence of an enlarged spleen and the probability of finding parasites in the blood is, of course, well known, and in this endemic area series, of the 362 children without enlarged spleens only 98 (27 per cent.) had positive blood findings, while of the 360 with enlarged spleens 200 (56 per cent.) had parasites in their blood. The relation, however, between the size of the spleen and the parasite findings is not so clear. When a table is prepared showing the size of spleen in association with which positive blood findings most commonly occur the latter are found to be grouped round two different sizes of spleens ; but further investiga-

TABLE III.

Showing the relation between size of spleen and presence of parasites in blood.

Size of spleen	Number	Percentage of all spleens	Number of positive bloods	Percentage of positive bloods in persons with palpable spleens
Just palpable ... ..	71	20	32	16
At costal margin ... ..	52	14	33	16.5
14 cms. from umbilicus ..	1	0	0	0
13 cms. " ..	6	2	3	1.5
12 cms. " ..	16	4	10	5
11 cms. " ..	25	7	11	5.5
10 cms. " ..	31	9	20	10
9 cms. " ..	39	11	16	8
8 cms. " ..	44	12	21	10.5
7 cms. " ..	36	10	27	13.5
6 cms. " ..	20	6	15	7.5
5 cms. " ..	12	5	6	6
4 cms. " ..	3		3	
3 cms. " ..	3		2	
3 cms. below umbilicus ...	1		1	



tion shows that these are the commonest sizes of spleen. In Table III the number of spleens of each size, the percentage they form of all the palpable spleens, together with the number of positive bloods and the percentage they form of positive bloods in persons with palpable spleens, are shown, and it will be seen that the two percentages agree closely; it is particularly noticeable that the largest spleens are not associated with diminished parasite findings.

*Age incidence of splenic enlargement.* The percentage of children with palpable spleens, shown in Table IV, remains practically constant throughout the age period examined; there does, it is true, appear to be a slight increase in the 11-12 group, but the increase and the number of children examined are not sufficiently large to justify the assumption that this is real and not merely due to the error of random sampling. The size of the spleen, shown in the same table, also appears to remain fairly constant.

TABLE IV.  
Showing the age incidence of blood and spleen findings in the endemic area.

Age	Number examined	Percentage with Positive blood	Percentage with palpable spleen	Average distance of enlarged spleen from umbilicus
3- 4 ... ..	23	43	48	9.5 cms.
5- 6 ... ..	145	46	52	10.4 cms.
7- 8 ... ..	211	39	48	10.2 cms.
9-10 ... ..	207	41	49	10.5 cms.
11-12 ... ..	117	40	55	10.4 cms.

Splenic measurements corrected by sitting height; spleens noted as 'palpable, but not reaching costal margin,' and 'at costal margin,' are included.

N.B.—The few children aged thirteen or over, and those of doubtful age, are omitted from this and all subsequent tables showing age incidences.

## (2) *Hyper-endemic Area.*

Of the 337 cases that occurred in the hyper-endemic area 244 (72 per cent.) had palpable spleens. Two hundred and sixty-five were aged ten or under and of these 183 (71 per cent.) had palpable spleens, so that the spleen index of Ross (1910) is 71, the average distance of the spleen from the umbilicus was 8.1 cms., considerably less than in the endemic area. The positions of the apices did not, however, show that division into two distinct groups which was

present in the endemic area, but were more or less evenly distributed about a mode at 8-9-10 cms. from the umbilicus.

*Age incidence of splenic enlargement.* In this area there is not the same relation between splenic enlargement on the one hand and age or parasite findings on the other, as in the endemic area. In Table V, in which blood and spleen findings are grouped in two-yearly age groups, the numbers being too small to allow of grouping in single year periods, it will be seen that the spleen rate falls from 86 per cent. during the 5-6 age group, to 60 per cent. at the age of ten, after which it returns to 81 per cent. in the 11-12 age group. Thus from being considerably higher than the parasite rate it drops to 20 per cent. below it and recovers to a point 20 per cent. above it again.

TABLE V.

Showing the age incidence of blood and spleen findings in the hyper-endemic area, and the average distance of the spleen from the umbilicus at different ages.

Age	Number examined	Percentage with positive blood	Percentage with palpable spleen	Average distance from umbilicus
3-4 ... ..	5	80	80	11.75 cms.
5-6 ... ..	80	75	86	8.5 cms.
7-8 ... ..	94	72	65	9.2 cms.
9-10 ... ..	86	80	60	8.7 cms.
11-12 ... ..	70	60	81	9.2 cms.

It is difficult to find an explanation for this drop after the age of six. It cannot be explained on the grounds of a difference in technique, as this was the same throughout and children of different age groups were examined side by side every day. The numbers examined in each age group seem substantial, but a repetition of the observation must be made on still larger numbers of children before the decrease and subsequent increase can be accepted as facts and have theories built on them.

*Relation between the size of spleen and age.* The size of the spleen, the variations in which are also shown in Table V, appears here to remain more or less constant at all the ages examined, excepting the 3-4 group, in which the number of children examined was too small to enable one to draw any conclusions from them.

*The relation between size of spleen and parasite findings.* In this area the relation between the presence of an enlarged spleen and the probability of finding parasites in the blood is not so great. Of the 93 without palpable spleens, 61 (66 per cent.) had positive bloods, while of the 244 with enlarged spleens, 183 (75 per cent.) were positive. The size of spleen showed the same relation to parasite findings as in the endemic area and it was again noted that the largest spleens were not associated with any diminution in the parasite findings, in fact, of the thirty-seven spleens projecting to within 6 cms. or less of the umbilicus, thirty-two (86 per cent.) were associated with an infected blood. The details of this are shown in Table VI.

TABLE VI.  
Relation between size of spleen and parasite findings.

Size of spleen	Number	Percentage of all spleens	Number of positive bloods	Percentage of positive bloods in persons with spleens
Just palpable ...	11	4.5	8	4
Costal margin ...	15	6	9	5
13 cms. from umbilicus ...	4	2	3	2
12 cms. „ ...	11	4.5	9	5
11 cms. „ ...	27	11	18	10
10 cms. „ ...	41	17	31	17
9 cms. „ ...	40	16	31	17
8 cms. „ ...	34	14	25	14
7 cms. „ ...	24	10	17	9
6 cms. „ ...	20	8	17	9
5 cms. „ ...	3	7	2	8
4 cms. „ ...	6		6	
3 cms. „ ...	1		1	
3 cms. below umbilicus ...	2		2	
4 cms. „ ...	2		2	
5 cms. „ ...	2		1	
8 cms. „ ...	1		1	

## II. EXAMINATION OF PERIPHERAL BLOOD

A single thin film was taken from the ear of each child and examined after staining with Giemsa. No film was pronounced negative until it had been examined for at least ten minutes; one thousand and fifty-nine films were thus examined and 542 (51 per cent.) showed malaria parasites.

### (a) *Endemic area.*

Of the 722 children examined here, 298 (41 per cent.) had infected bloods. Benign Tertian, Quartan and Malignant Tertian parasites were all seen, the latter by far preponderating. Below are shown the number of each species, the three double infections being counted under both parasites :—

Malignant tertian	...	...	...	...	...	251	83 per cent.
Quartan	...	...	...	...	...	41	14 „
Benign tertian	...	...	...	...	...	5	2 „
Infections diagnosed on pigmented leucocytes only	...	...	...	...	...	4	1 „

*Age incidence of malaria findings.* Table VII shows the percentage of children infected at each age group from 3 to 12. This remains steady at approximately 40 per cent. during the whole of the age period examined.

TABLE VII.

Showing the age incidence of parasite findings in the endemic area.

Age								Number examined	Percentage with positive blood
3-4	...	...	...	...	...	...	...	23	43
5-6	...	...	...	...	...	...	...	145	46
7-8	...	...	...	...	...	...	...	211	39
9-16	...	...	...	...	...	...	...	207	41
11-12	...	...	...	...	...	...	...	117	40

### (b) *Hyper-endemic area.*

Of the 337 children whose blood was examined, 244 (72 per cent.) had malaria parasites in their blood, Quartan and Benign tertian

infections being slightly more common than in the endemic area, the actual numbers being :

Malignant tertian	...	...	...	...	...	191	78 per cent.
Quartan	...	...	...	...	...	46	19 „
Benign tertian	...	...	...	...	...	6	2 „
Infections diagnosed on pigmented leucocytes only	...	...	...	...	...	1	1 „

The age incidence of these infections, which is seen in Table VIII, remained stationary, as in the endemic area, till the age of ten, the rate ranging round 75 per cent., but then showing a sharp fall to 60 per cent.

TABLE VIII.

Showing the age incidence of parasite findings in the hyper-endemic area.

Age	Number examined	Percentage with positive blood
3-4	5	80
5-6	80	75
7-8	94	72
9-10	86	80
11-12	70	60

### III. THE INTENSIVE EXAMINATION OF CHILDREN

Although the parasite rate and spleen rate in the endemic area were both below 50, it was suspected that a much larger percentage of the children than this was infected with malaria. A school was therefore chosen, in which the children were under good discipline, and fifty-six boys were examined daily (or until found positive), not more than seven examinations, however, being made of any child. Of these fifty-six, five proved to have taken quinine for 'fever' within three days of the commencement of examination, one refused examination after the third time and one remained absent after the second examination; these seven were all disqualified and do not appear in the following figures. Of the remaining forty-nine the results for a single examination were :—

Number with positive blood film	...	...	...	23	47 per cent.
Number with enlarged spleen	...	...	...	24	49 „

The subsequent examinations revealed malaria in nineteen more of the children, making a total of forty-two (85·7 per cent.) with positive blood films. One of these nineteen had also been taking quinine within a week of examination and was diagnosed on pigmented leucocytes alone. Of the seven who remained negative after seven consecutive examinations, two had enlarged spleens, two had temperatures of 99·4° F., two had temperatures of 99° F., one only being entirely normal, with a temperature of 98·6° F., no palpable spleen and a consistently negative blood film. Of these 49 children, therefore, 48 (98 per cent.) showed definite evidence of illness, and in 42 (86 per cent.) this illness was definitely malaria.

The age grouping of these children was as follows :—

Age							Number examined	Positive first examination	Positive at any examination
5-10	...	...	...	...	...	...	33	16 = 48 %	29 = 88 %
11-16	...	...	...	...	...	...	17	6 = 35 %	13 = 76 %

The figures are too small to draw any definite conclusions as to the age grouping of these infections, but suggest that the older group of children is more lightly infected as is shown by either single or repeated examinations.

A similar examination was made of the children attending School in Murray Town, a small village outside Freetown, in which a single examination gave results comparable to the endemic area already described ; thirty-seven children were here examined, the results for a single examination being :—

Number examined	...	...	...	...	...	37
Number with positive blood film	...	...	...	...	...	18 = 49 per cent.
Number with palpable spleen	...	...	...	...	...	17 = 46 %

The six subsequent examinations showed parasites in another thirteen, so that thirty-one (84 per cent.) were finally found infected. Of the remaining six, one had a temperature of 100° F. and an enlarged spleen, two had temperatures of 100° F., one had a temperature of 99·8° F., and two had temperatures of 99·6° F., so

that the entire thirty-seven were suffering from some disease, in all probability malaria.

The combined figures for the two schools examined in this intensive way are :—

Number examined	...	...	...	...	...	86	
Number with palpable spleen	...	...	...	...	...	41	= 48 per cent.
Number with positive film on first examination	...	...	...	...	...	41	= 48 „
Number with positive film at any examination	...	...	...	...	...	73	= 85 „

while, of the remaining thirteen, twelve had either a high temperature or a palpable spleen. It would appear from the above that the true difference between a hyper-endemic and an endemic area is not that a much higher percentage of children are infected in the former, but that the severity of infection is greater, probably due to a higher inoculation rate. In both districts the children are continually suffering from malaria, but in the endemic area the child has the disease under control for a large part of the time, a decrease in the parasite index being produced either by a decrease in the inoculation rate, or by an increase in the immunity of the child, and not by a decrease in the number of children infected.

The absence of an enlarged spleen probably signifies relatively light infection, one, that is, in which the child's peripheral blood does not constantly contain malaria parasites, but gives little clue as to whether or not they will be seen on repeated examination ; thus, of the forty-one with enlarged spleens in these two schools, twenty-six (63 per cent.) showed parasites in a single examination and on subsequent examination thirty-eight (93 per cent.) were positive. Amongst the forty-five without enlarged spleens only fourteen (31 per cent.) were positive on the first day, but the number was increased on subsequent examination to thirty-five (78 per cent.).

*Concentration of malaria round Anopheline breeding places.* The restriction of malaria to the immediate vicinity of anopheline breeding places has been pointed out by Ross (1910) and others, while Blacklock (1921) and Blacklock and Evans (1926) have shown the dependence of the anophelines in Freetown on the streams. In order to confirm these and the statements that have previously been made about the cause of difference in the severity of infection in the two districts, two areas were chosen and the condition of the blood of all children living in them noted.

The first area (area *A*) was a square in the centre of the town, bounded by Oxford Street, Garrison Street, and Pademba Road, Percival Street and Wilberforce Street, on a gentle slope, well drained and no part of it less than a quarter-of-a-mile from the nearest breeding place in the spot map shown in Blacklock and Evans' (1926) paper. The second area (area *B*) consisted of a number of short streets in the region of Sanders Brook, none of them extending to more than a quarter-of-a-mile away from the nearest breeding place in the map. These two areas are not far separated, the Eastern border of one approaching within two hundred yards of the Western border of the other. Sixty-six children from the first area were examined and seventeen (26 per cent.) had malaria parasites in their blood ; whilst of 78 children who were seen from the district along the bank of the stream, 49 (63 per cent.) were positive. The map of Blacklock and Evans (1926) showing anopheline breeding places, is reproduced (by permission) in fig. 3, with areas *A* and *B* marked on it.

#### IV. THE EXAMINATION OF TEMPERATURES

The mouth temperature of most of the children (unfortunately not all, as this was started a week or so late) was taken with an N.P.L.\* stamped thermometer which, though not locally tested against a standard thermometer, gave normal readings in Europeans.

Of the one thousand and seven children whose temperatures were taken, in the whole town only 61 (6 per cent.) were below 99° F., 528 (52 per cent.) were between 99° and 99·9° F., while the remaining 418 (42 per cent.) were 100° F. or over. This preponderance of raised temperatures has been noted before by Butler (1913), in Freetown, and by Magill (1923), in Accra and Seccondi ; both of these observers noted the apparent lack of relation of the high temperature to the finding of malaria parasites in the blood, and the latter remarks that 98·4° F. can hardly be regarded as the normal for the African School child.

Tables IX and X below show the incidence of the various temperatures.

\*National Physics Laboratory.



TABLE IX.

	Endemic Area		Hyper-endemic Area	
	Number	Percentage	Number	Percentage
Number of temperatures taken ... ..	672	...	335	...
Below 99° ... ..	60	9	1	0'3
99-99'9° ... ..	384	57	144	43
100° and over ... ..	228	34	190	57

TABLE X

Showing the age of incidence temperatures of 100° F. and over.

## A. ENDEMIC AREA.

Age ... ..	3	4	5	6	7	8	9	10	11	12
No. of temperatures taken	2	20	50	75	99	98	104	101	81	30
Percentage 100° F. or over	50	50	30	43	34	45	33	32	26	17

## B. HYPER-ENDEMIC AREA.

Age ... ..	3	4	5	6	7	8	9	10	11	12
No. of temperatures taken	3	2	16	64	51	41	48	39	40	29
Percentage 100° F. or over	...	...	62	58	61	63	67	59	55	21

It will be seen that in each area the percentage with high temperatures varies about a mean between the ages of 5 and 10 years, 60 per cent. in the case of the hyper-endemic area, 40 per cent. in the endemic area, and then shows a marked decrease, coinciding with the decrease in the parasite index.

There was no observed relationship between the occurrence of a temperature of 100° F. or over, and the finding of malaria parasites or a palpable spleen, as is shown below :—

TABLE XI

Showing the relation between blood, spleen and temperature findings.

	Percentage with temperature of 100° <sup>1</sup> or over	
	Endemic area	Hyper-endemic area
Children with positive blood ... ..	34	55
Children with negative blood ... ..	34	62
Children with palpable spleen ... ..	35	57
Children without palpable spleen ... ..	33	56

Despite the apparent lack of relationship between parasite and spleen findings and the temperature, shown here and in Butler's and Magill's work, I consider that the normal temperature of the African school child is the same as that of the European and that the high temperature is due to malaria, for the following reasons :—

(1) It is the experience of medical practitioners practising medicine locally that the administration of quinine to children rapidly brings the temperature to 98·4° F. or thereabouts, and that this is the normal temperature in the adult.

(2) The incidence of the high temperatures coincides with that of parasite findings; thus in the less heavily infected area, with a parasite rate of 41 per cent., 34 per cent. had temperatures of 100° F. or over; in the hyper-endemic area, parasite rate 72 per cent., 57 per cent. had these temperatures. Dr. Magill (1923), working in Accra, examined 288 children with a parasite rate of 19 per cent., and only 40 (14 per cent.) had temperatures of this height. Butler, unfortunately, does not give the number of children with temperatures of 100° F.

(3) The changes in the incidence of high temperatures at different ages are very similar to those in the parasite rate.

We may therefore take it that the high temperatures in school children living in malarious countries is due to malaria, that the percentage of children with these temperatures serves as an indication of the parasite and spleen rates, but it cannot be assumed that

because any individual child has a temperature of 100° F. or over that it therefore has parasites in the peripheral blood at the time.

*Malaria amongst Mulattos.* The frequency of enlarged spleens in mulatto children was noticed by Magill (1923), in Accra, where amongst the sixty mulattos examined, 59 per cent. had enlarged spleens, against 16 per cent. amongst native children.

In this examination only thirty-three mulattos were seen, nineteen boys and fourteen girls. Twelve came from the hyper-endemic area, all of these had palpable spleens, the average distance from the umbilicus being 7·8 cms. Nine (75 per cent.) had temperatures of 100° F. or over, and five (42 per cent.) had positive bloods. Twenty-one lived in the endemic area, of whom seventeen (81 per cent.) had palpable spleens, on the average 9·1 cms. distant from the umbilicus, six (29 per cent.) had positive bloods, and of the twenty whose temperature was taken, in nine (45 per cent.) it was 100° F. or over.

It would appear from the above that in both areas the mulatto children have higher temperature rates and spleen rates, larger spleens and lower parasite rates than the native children. The number of them examined, however, is too small to permit of any reliable conclusions being drawn.

### SUMMARY

1. One thousand and fifty-nine children, aged 3 to 12, in the schools of Freetown, Sierra Leone, were examined for the condition of their blood, spleen, and temperature, between July, 1925, and March, 1926.

2. Abdominal measurements were made of all children and a method of correction of splenic measurements for the size of the child, similar to that of Christophers (1924a) was devised.

3. It was found possible to divide the town into two areas, a hyper-endemic area in close proximity to the breeding places of *A. costalis*, and an endemic area remote from them.

4. The spleen rates in the endemic and hyper-endemic area respectively were 50 and 72, and the parasite rates 41 and 72.

5. In the endemic area there was no sign of diminution of the spleen or parasite rate during the age examined; in the hyper-

endemic area, however, the parasite rate diminished at the age of twelve.

6. It was shown by the intensive examination of 86 children living under endemic conditions that although only 48 per cent. had parasites in their peripheral blood at a single examination, yet at least 85 per cent. were suffering from malaria.

7. The cause of the varying severity of malaria in different districts of the town was demonstrated by selecting two areas, one containing numerous anopheline breeding places, one only a few or none, and comparing the condition of the children in each.

8. Thirty-three mulattos were seen and it is suggested that possibly they re-act to malarial infection in a different manner from pure negroes.

9. The temperature of over 1,000 children was taken and it was found that a temperature conforming to a normal of 98.4° F. was a comparative rarity. Evidence is given which goes to prove that this is not due to the African's normal temperature being higher than that of the European, as suggested by some, but is a pathological rise due to malaria. It is possible that in the elevation of the temperature we have a more accurate indication of malarial infestation in endemic areas than in either the parasite or the spleen rate.

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## MAP OF FREETOWN

Showing anopheline breeding places, reproduced by permission, from Blacklock and Evans (1926). The two areas, A and B, marked on it are those referred to on page 257. The parasite index of B, where anopheline breeding places are numerous, was 63 per cent. and that of A, which is devoid of them, was 26 per cent. The hyper-endemic area consists of area B and the district to the east of it; the endemic area is to the west of area B.





# A NEW LARVA OF *OESTRUS (GASTROPHILUS)* FROM ZEBRAS

A. W. N. PILLERS

AND

A. M. EVANS

(Received for publication 21 May, 1926)

## PLATE XX

The species which forms the subject of the present paper is described from four batches of bots, three of which were collected from zebras in Rhodesia, by Professor Yorke, in 1912. The fourth consignment was taken from the stomach of a zebra, which was sent to one of the authors by Professor F. T. Hobday, in February, 1925. All three lots of Professor Yorke's material contained in addition specimens of *O. pecorum* Fabr., and *O. ternicinctus* Ged., two lots contained also larvae of *O. gedoelsti* Rod. and Beq., and Brauer's No. 2 and No. 3 *Oestrus* larvae from *Equus bohmi*, and in the stomach of Professor Hobday's zebra were found numerous larvae of *O. ternicinctus*, and also specimens of *O. gedoelsti* and Brauer's No. 2 larva from *Equus bohmi*.

The specimens here described occurred in comparatively small numbers, but showed no variation in the important characters which distinguish them from the larvae of the known species of *Oestrus*.

Mr. W. H. Potts, B.A., formerly of the Liverpool School of Tropical Medicine, who examined the material before leaving for Tanganyika Territory, in 1925, came to the conclusion that it was quite distinct, and we have no hesitation in describing it as a new species.



*Third stage larva :*

Body cylindrical, flattened ventrally, the anterior end sub-conical, the posterior rounded or somewhat truncate ; the segments increasing in width from before backwards to the eighth and ninth ; the tenth and eleventh decreasing slightly and the twelfth abruptly narrower than the preceding. Third segment markedly projecting dorsally. Colour of integument varying ; whitish, creamy, ochraceous or pale ochraceous-brown. Spines terminating in a fine point.

*Length :* 13 to 15 mm. *Greatest width :* about 6 mm.

*Spinulation. Ventral series :* third and fourth segments unarmed ; fifth to ninth segments each with a single uninterrupted row of spines ; tenth segment with a single row of spines, somewhat variable in number, but usually about eight, the distance between the median pair greater than that between other adjacent pairs ; eleventh segment variable, either : unarmed ; with a reduced number of spines, widely interrupted ; or with a complete row. *Dorsal series :* third and fourth segments unarmed ; fifth to ninth segments with a single interrupted row of spines ; tenth segment unarmed or with a few (about one to four) spines at each side ; eleventh segment unarmed. *Lateral series :* variable and somewhat irregular (see Pl. XX, fig. 3). No interruption between ventral and lateral series ; interruptions present or absent between dorsal and lateral series.

This larva is most closely related to that of *O. veterinus* Clark (*O. nasalis* Brauer) but differs markedly from it in the complete absence of spines on the third and fourth segments.

*Co-types :* Eight larvae taken from a zebra, Ngao, N.E. Rhodesia, 26.v.1912, Professor Warrington Yorke, M.D. ; other material from zebras, Rhodesia, 23.iii.1912 and 20.v.1912, Professor Yorke, and from a zebra of the Burchell Group imported to England from Pretoria, Professor F. T. Hobday, C.M.G., F.R.C.V.S.



## EXPLANATION OF PLATE XX.

*Oestrus meridionalis* sp.n. ; third stage larva, enlarged seven times.

Fig. 1. Dorsal view.

Fig. 2. Ventral view.

Fig. 3. Lateral view.

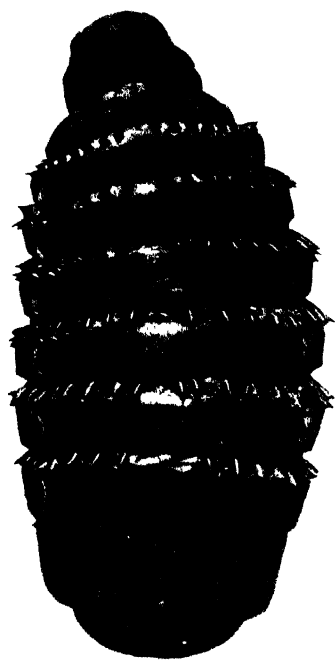
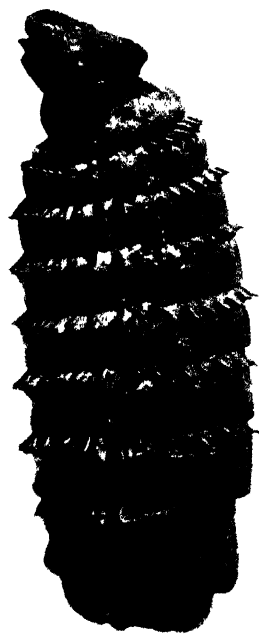
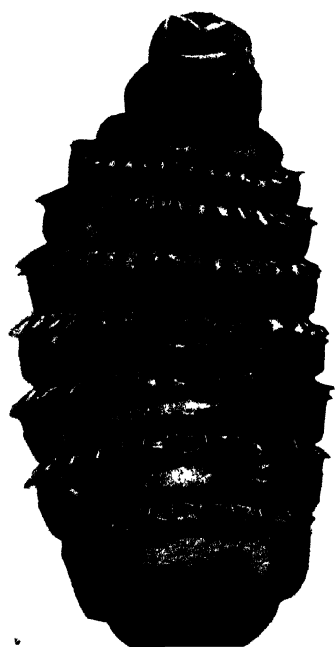


FIG. 1





# THE ACTION OF CARBON MONOXIDE ON CERTAIN BLOOD PROTOZOA

BY

V. DOGIEL

AND

T. FEDOROWA

(LENINGRAD)

*(Received for publication 25 May, 1926)*

The general idea, which led us to begin the experiments described in this paper, was to elucidate the question, if carbon monoxide cannot be proved to exert a certain toxic influence on different blood-parasites (*Haemosporidia*, *Trypanosoma*, etc.). It is a well-known fact that the inhalation even of very small quantities of CO not only produces a poisonous effect on the higher terrestrial vertebrates (Mammalia, Aves), but at the same time deprives the erythrocytes of these animals of their capacity to absorb the oxygen contained in the lungs. Therefore it is to be presumed that the quantity of oxygen contained in the blood, and especially in the erythrocytes, of the vertebrates poisoned by CO, is considerably smaller than that of normal animals. If so, then it is permitted to surmise, *a priori*, that the change of oxygen-content in the erythrocytes may prove deleterious for blood-parasites included therein. If, on the other hand, the dose of CO fatal for the blood-parasites can be supported without serious damage by their host, then a new departure in the methods of struggle against blood-parasites will be opened. The main feature of the action on the parasites, proposed by us, consists in injuring their respiratory functions. The fact that *Plasmodium*, *Karyolysus*, *Babesia*, etc., choose the erythrocytes as their abode, seems to indicate that these Protozoa are in constant need of oxygen, so that a lack of this gas must influence their vitality. In this respect our experiments differ entirely from the procedure recently employed by Cleveland (1925) for disinfection of the gut-contents of termites, frogs, etc., and for the killing of their intestinal Protozoa. Cleveland, as appears from his very interesting papers, used, for his purposes, the oxidising action of oxygen, keeping the test-animals in sealed jars with pure oxygen at  $3\frac{1}{2}$  atmospheres pressure.

Our work was begun quite independently of the American author, in the month of March, 1925, but owing to the lack of material, was protracted until the autumn of the same year.

In the vicinity of Leningrad there are very few animals harbouring blood-parasites, so that we were obliged to use common lizards (*Lacerta vivipara*) infected by the Haemogregarines of the genus *Karyolysus*. In addition, a few experiments were conducted with the rice-bird (*Padda oryzivora*) procured from abroad and infected by *Haemoproteus oryzivorae*. Even this material was not too abundant, because a great percentage of the lizards procured was free from parasites. During the whole summer we were able to obtain about 200 lizards, 110 being young, and the remainder adult. The young ones (of the brood of the same year) were never infected, whereas sixteen of the adult lizards were more or less heavily infected with *Karyolysus*. Of this number a certain number perished at the beginning of the experiments owing to insufficient care in handling them, so that the number of successfully conducted experiments became relatively small. But as we are by no means certain that the circumstances will permit us to continue our work during the summer of 1926, and as they appear to give some positive results, we take the liberty of publishing a preliminary note of our results.

The experiments were conducted thus:—The infected lizards were put under a glass bell-jar, which was fixed on a board by means of a rubber ring, and the edge of the jar was hermetically sealed by a thick varnish. The capacity of the bell-jar in different experiments varied from  $1\frac{1}{2}$  to 10 litres, but it was always such that the animals could survive with no visible deleterious effects when the jar contained normal air. Through a hole in the board CO was introduced by means of some tubing. A thermometer, if needed, was placed under the jar at the beginning of the experiment.

In experiments with birds, ordinary room-temperature (16-17° C.) can be used, because the birds are highly susceptible to the action of CO. The lizards, on the contrary, can endure a very long sojourn in CO of high concentration (for instance, in air containing 7 per cent. of CO) without being poisoned by it. This circumstance obliged us to combine the action of CO with that of high temperature, which combination proved to be of greater effect. The animals were kept in the jar until marked symptoms of suffocation (convulsions,

acceleration of the respiratory movements, closing of the eyes, etc.) were observed. Then the jar was removed and the animal was allowed to recover (for five to fifteen minutes), when the experiment was renewed. The continued repetition of the experiment was prompted by the idea that for successful action on the blood-parasites, the whole amount of blood of the animal must come in contact with CO. The amount of time passed by the animals in the atmosphere of CO differed greatly in different experiments. Thus birds survive exposure to CO, even of low concentration only, three to fifteen minutes, while lizards survive for several hours.

To estimate the effect of the treatment with CO, blood smears were taken just before and after each experiment. In lizards the blood was taken by cutting off a small piece of the tail, in birds the blood was got from the leg. The cut was treated with iodine to prevent bacterial infection. The smears were stained by Giemsa, and the number of parasites present in a certain number of microscopic fields (Zeiss Ocular 7, Obj. Hom. Immersion 2 mm.) was carefully counted.

*Lizards.* The erythrocytes of *Lacerta vivipara* contain the merozoites of *Karyolysus* in the form of small worm-like bodies. The a-sexual reproduction (schizogony) of *Karyolysus* proceeds not in the blood, but in the internal organs (spleen). This circumstance evidently can affect the results of the experiments, as the number of the parasites in the blood can be increased at the expense of the spleen-parasites; but in cases where the number of parasites in the blood is, nevertheless, markedly reduced after the experiment, the experiment may be considered to be positive.

The first experiments with lizards showed that they could endure an atmosphere of CO for twenty-seven to forty-eight hours, without experiencing any visible discomfort. The action of CO being too slight, we decided to combine the action of the gas with that of a high temperature produced by direct sunlight. The jars were exposed to the direct influence of the sun's rays. The temperature rose to 38°-42°. The lizards invariably died after thirty to forty-five minutes. If taken out of the jar in time, they recovered, although sometimes it was necessary to have recourse to artificial respiration, and it was necessary to be sharply on the look-out for suspicious symptoms, otherwise the animal was past recovery. After several minutes of respite the experiment was resumed.



The following records were obtained :—

## LIZARD NO. I.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the first experiment ...	200	133	22 July	—
2. After the first experiment ...	200	34	22 July	2 hours 30 minutes under the bell-jar (with short intervals) in 1 per cent. CO. About half of the time under direct sun-rays.
3. Before the second experiment...	200	13	24 July	—
4. After the second experiment ...	200	8	24 July	2 hours under the bell-jar (with short intervals).
5. After a long period of life in normal conditions ...	200	5	15 Aug.	—

Lizard No. I died at the end of August, of unknown causes, but to the end of its life it continued to show only a minimal number of parasites.

## LIZARD NO. II.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the experiment ...	200	444	25 July	—
2. After the experiment ...	200	226	25 July	2 hours (with short intervals) in 1 per cent. CO. Evening, the sun not very hot.
3. After the second experiment ...	200	150	26 July	35 minutes in 1 per cent. CO. Sun very hot; the lizard died after the experiment, not withstanding the artificial respiration.

## LIZARD No. III.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the experiment ..	300	272	23 Aug	
2. After the experiment ...	300	87	23 Aug	45 minutes (with one interval) in 1 per cent. CO

## LIZARD No. IV.

	Number of micro. fields examined	Number of parasites found	Date	Remarks
1. Before the experiment ...	200	1,026	29 July	
2. After the experiment . ...	200	572	30 July	45 minutes in CO of very low concentration (0.25 per cent) Sun very hot, the animal was restored to life only by means of artificial respiration
3. Before the second experiment...	200	666	10 Aug.	
4. After the second experiment ...	200	372	10 Aug	2 hours (with interval) in 2 per cent CO The animal died at the end of the second experiment

The data show that in lizards I-IV the number of blood-parasites was greatly reduced by the treatment, although a total extermination of *Karyolysus* could not be attained. In the case of lizard No. I, the reduction of the number of parasites attained continued for a long period, so that the parasites in the internal organs seem to have been destroyed.

Experiments carried out by exposure to heat alone gave a negative result, and it appears to be the case that only the combined effect of the high temperature and CO was effective.

*Birds.* A specimen (*Padda oryzivora*) was exposed to 0.5-1 per cent. CO, at a temperature of 15-17° C., in a jar of 10 litres

capacity, for two-and-a-half to three hours (with several short intervals) during one experiment. Such experiments were repeated with one bird seven different times, till it died, but no reduction of the number of gametocytes could be observed. It is interesting to compare these results with the experiments of Cleveland, on the toxicity of oxygen to the intestinal Protozoa. Cleveland has, so far, likewise failed to affect the Protozoa of the higher vertebrates possessing a constant blood-temperature, while he succeeded in destroying the parasites of the frog.

Although our experiments are far from conclusive, however, the marked reduction in the numbers of parasites in the case of lizards treated by CO is evident and calls for further research.

# NOTES ON SOME NEMATODES IN THE MUSEUM OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

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*(From the Parasitological Department of the Liverpool School of  
Tropical Medicine)*

*(Received for publication 8 June, 1926)*

## I. *ECHINURIOIDES PLECTROPTERI* n.gen., n.sp.

Host : Spurwinged goose, *Plectropterus* sp. Locality : Northern Nigeria.

The worms are of small size, the two males measuring 4.2 and 4.4 mm. in length, by 0.12 and 0.09 mm. in greatest breadth, respectively.

The body tapers slightly both anteriorly and posteriorly, the tapering tail ending in a blunt point. The cuticle is transversely striated throughout, the striations occurring at intervals of about 7 $\mu$ .

The cervical papillae are slightly in front of the nerve ring, about 135 $\mu$  from the anterior extremity ; the excretory pore is slightly more anterior.

The cuticle is provided with four longitudinal rows of spines which lie laterally, one on each side of the lateral lines. They commence a little behind the anterior end of the oesophagus at a point about 55 $\mu$  from the anterior extremity of the worm, and become more concentrated in front of, and behind, the anus, where they have the appearance of papillae.

The cervical cuticle is provided with four cordons ; these appear to arise from cuticular thickenings at the base of the lateral lips and run backwards, one on each side and close to the lateral lines, to terminate about 100 $\mu$  from the anterior extremity ; they are extremely fine and slender and do not anastomose on the lateral surfaces posteriorly.

The head is provided with four lips, two lateral, and a dorsal and a ventral. The former measure 13 by  $15\mu$ , and the latter, 13 by  $26\mu$ , in height and maximum breadth, respectively. The dorsal and ventral lips are each provided with a pair of papillae at their bases. The mouth leads into a comparatively short vestibule about  $22\mu$  in length; the dorso-ventral diameter is distinctly greater than the lateral. The oesophagus measures 1.3 mm. in length and  $55\mu$  in maximum breadth, and is divided into two parts,

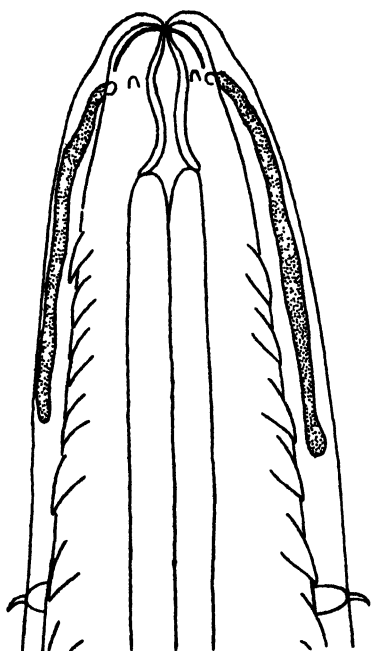


FIG. 1

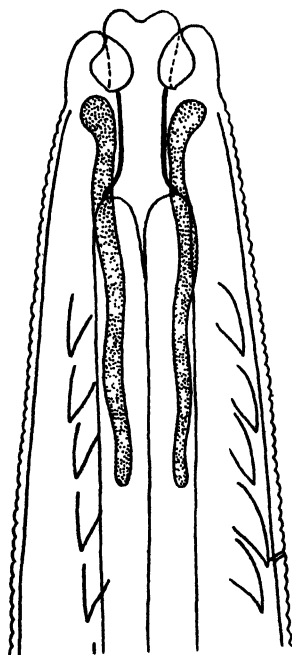


FIG. 2

FIG. 1. *Echinurioides plectropteri*. Anterior extremity. Ventral view.  $\times 650$ .

FIG. 2. *Echinurioides plectropteri*. Anterior extremity. Lateral view.  $\times 650$ .

the anterior measuring about  $364\mu$ . The ratio between the length of the oesophagus and that of the worm is 1 to 3.4. The anus opens at a point  $133\mu$  from the tip of the tail, and the width of the body at the anus is about  $55\mu$ .

The spicules are unequal; the larger measures about  $851\mu$  and the shorter about  $145\mu$  in length.

In the possession of cuticular cordons and four longitudinal rows of spines this worm resembles the genus *Echinuria*, but it differs markedly from this genus in not possessing caudal alae, in that the cordons do not anastomose posteriorly, and in having four lips.

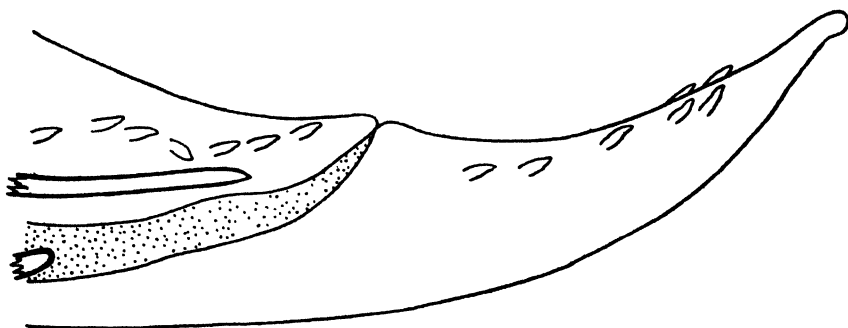


FIG. 3. *Echinurioides plectropteri*. Caudal extremity of male. Lateral view.  $\times 487$ .

These differences, it is considered, are sufficiently distinctive to warrant the erection of a new genus and the generic name *Echinurioides* is suggested, with *E. plectropteri* as the specific name.

## 11. *AMPLICAECUM CAUSI* n.sp.

Host : *Causus rhombatus*. Position : Small intestine. Locality : Northern Nigeria.

The worm is of medium size, 15.5 to 23 mm. in length, by 0.52 to 0.65 mm. in thickness. The body gradually tapers towards the head, while posteriorly, in both sexes, the tail has the form of a short sharply-pointed cone. The excretory pore opens at a distance of about 500 to 686  $\mu$  from the anterior extremity of the worm, and the cervical papillae are found slightly more posterior at distances of 530 to 748  $\mu$  from the same point. There is a well-marked neck, the width of which is from 155 to 170  $\mu$ ; the cuticle is provided with very fine striations.

The three lips are more or less rectangular in shape and measure about 135 by 148  $\mu$  in height and maximum breadth, respectively. The dorsal lip is provided with two horseshoe-shaped papillae; each lateral lip possesses only one papilla, the outline of which is complete;

the pulp is massed into two large equal lobes with evenly-rounded extremities. Dentigerous ridges are present on the margins of the lips which they follow closely throughout. The small interlabia measure about  $44\mu$  in height.

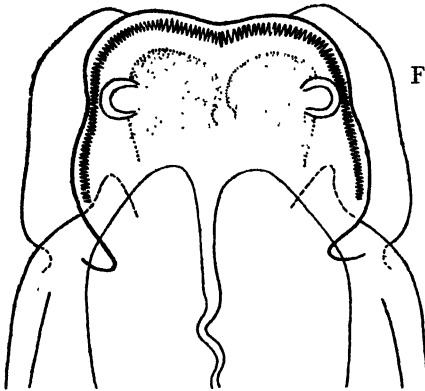


FIG. 4

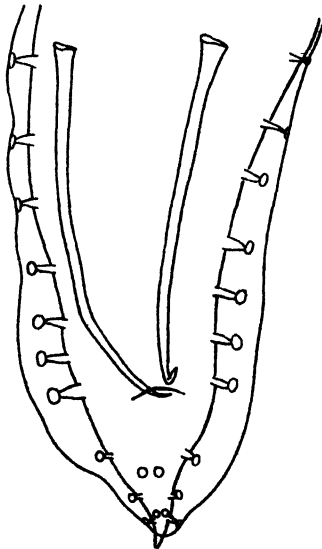


FIG. 6

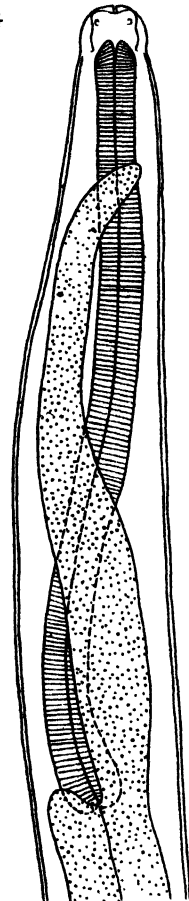


FIG. 5

FIG. 4. *Amplicascum causi*. Head. Dorsal view.  $\times 256$ .

FIG. 5. *Amplicascum causi*. Oesophageal portion. Dorsal view.  $\times 90$ .

FIG. 6. *Amplicascum causi*. Caudal extremity of male. Ventral view.  $\times 90$ .

The oesophagus measures 2.3 to 2.8 mm. in length, by about 0.14 mm. in breadth, and the ratio between the length of the oesophagus and that of the worm is from 1 to 6 or 8.5. A large intestinal caecum is present, lying dorsally to the oesophagus, terminating at a point varying from 450 to 936 $\mu$  from the anterior extremity.

The males measure 20 to 23 mm. in length, by 0.5 to 0.6 mm. in thickness; the anus opens at a point about 208 $\mu$  from the caudal extremity, and the width of the body at the anus has approximately the same measurement. Well-developed caudal alae are present. There are about thirteen pairs of preanal, and five pairs of postanal papillae arranged as shown in Fig. 6. The spicules are equal and taper slightly and have a length of 774 to 803 $\mu$ .

The females measure 15.5 by 0.5 mm. to 20.6 by 0.6 mm. The vulva opens anterior to the middle of the body, about 6.3 to 8.1 mm. from the anterior extremity. The caudal extremity is about 176 $\mu$  from the anus, at which point the breadth of the body has approximately the same measurement. As none of the females were gravid the dimensions of the eggs cannot be given.

Reference to the literature relating to this genus shows that no species has yet been recorded from a snake.

*A. colorum* (Baylis, 1919) from an eagle differs in length, in the absence of papillae from the dorsal lip, in the shape of the lips, and in the position of the vulva.

*A. africanum* Taylor, 1924, from a toad is also a longer worm and the ratio between the length of the oesophagus and the length of the worm is 1 to 11, as compared with a ratio of 1 to 8.5 in the specimens here described. Taylor's species also differs in having only three postanal papillae, somewhat larger lips, and in the possession by some specimens of a second intestinal caecum.

*A. gedoelsti* Yorke and Maplestone, 1926, also from a toad, possesses spicules which are longer than those in the specimens here described and is peculiar in that the pulp of the dorsal lip sends out lateral and apical processes; the female worms are of a much greater length.

*A. varani* Baylis and Daubney, 1922, differs greatly in possessing thirty-two pairs of preanal papillae, two papillae on each lateral lip, in the absence of caudal alae, and in having much shorter spicules.

*A. involuta* (Gedoelst, 1916) from a chameleon differs chiefly in the



males' being considerably shorter in length and possessing longer spicules; there are only three postanal papillae on the tail of the male.

It is thought that the differences enumerated here are sufficient to justify the erection of a new species for the worm in question and the specific name *Amplicaecum causi* is suggested.

### III. *TRICHURIS OVIS* IN *DAMILISCUS TIANG*

Several specimens of a worm which is indistinguishable from *T. ovis* were taken from the caecum of a Topi shot by the author, near Masaka, Uganda.

This nematode does not hitherto appear to have been recorded from this antelope.

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# OBSERVATIONS ON *ENTAMOEBA* *HISTOLYTICA*

## I. DEVELOPMENT OF CYSTS, EXCYSTATION, AND DEVELOPMENT OF EXCYSTED AMOEBAE, *IN VITRO*

BY  
WARRINGTON YORKE  
AND  
A. R. D. ADAMS

(Received for publication 24 June, 1926)

### PLATES XXI-XXIV

For many years it has been generally accepted that the cysts of *Entamoeba histolytica* will hatch only after they have been subjected to the action of the gastric and pancreatic juices, or to that of the pancreatic juice alone. Study of the literature suggests that this hypothesis is probably mainly based on the observations of Ujihara (1914), of Penfold, Woodcock, and Drew (1916), of Chatton (1917), and of Cutler (1919). Ujihara records that cysts after incubation with gastric juice for 24 hours at 37° C. remained for the most part undigested, but that pancreatic juice was much more active. Penfold, Woodcock, and Drew write as follows: 'As excysting agents we have tried pepsin in an acid medium, bile, and pancreatic extract, either alone, consecutively, or together, as appeared indicated, but the only success we have had has been with pancreatic extract used alone.' Cutler states that 'if a solution of "liquor pepticus" is first allowed to act on the cysts for a short time, followed by a similar solution of "liquor pancreaticus," a very large proportion of the treated cysts react.' Chatton's experiments were of a different type, but permit of similar conclusions; he fed cats with *E. histolytica* cysts, and sacrificed the animals after periods varying from 3½ to 17 hours; careful examination of the contents of different portions of the alimentary canal caused him to draw the conclusions that cysts pass through the stomach without alteration, except for the digestion of chromidial bars, and that excystation takes place in the small intestine.

It is remarkable that these workers have either overlooked or ignored the very interesting paper of Darling (1913). Chatton, it

is true, refers to his work, but offers no comment upon it. Darling describes the gradual disappearance of cysts from moist chamber preparations of heavily-infected faeces, and also the development of amoebulae within the cysts, and their emergence. This statement is so definite and based upon apparently such careful observations that it is curious it should have escaped comment from those who later worked on the same subject. Probably there are several reasons for this, most influential of which was the general pre-conception that the cysts must be swallowed before they can develop; another consideration was, doubtless, the possibility that Darling's preparations were—as so often happens in the tropics—contaminated with free-living amoebae.

However this be, the fact remains that Darling's work seems to have been more or less ignored and the view generally accepted that *Entamoeba histolytica* only excysts when it has been swallowed and subjected to the influence of the digestive juices. Dobell and O'Connor (1921), in their book on *The Intestinal Protozoa of Man*, after considering previous work on the subject conclude that 'it is certain that the cysts never hatch in the colon, where they are found, or outside the body.'

In 1924, however, an important piece of work was published by Sellards and Theiler, which indicated that *Entamoeba histolytica* cysts would hatch when injected intra-rectally in kittens, and their work, which was very carefully controlled, has quite recently been confirmed by Hoare (1925).

This observation is naturally of considerable importance and re-opens the whole subject regarding the conditions necessary for the excystation of *Entamoeba histolytica*, and possibly has some bearing on the factors concerned in relapse production in amoebic dysentery.

With the object of throwing further light on the matter we have conducted, during the past six months, a large number of experiments, the nature of which is described below.

The work commenced with the inoculation of a couple of tubes of Locke-egg-serum medium (Boeck, 1924, and Boeck and Drbohlav, 1925) with a small amount of a human faeces containing numerous cysts of *Entamoeba histolytica*, and incubating at 37° C. On examining the tubes next day, we were surprised to find large numbers of vegetative amoebae morphologically indistinguishable

from *Entamoeba histolytica*. From these tubes others were sub-inoculated, and it was found that the strain was just as readily maintained for many generations, as were those originating from the vegetative forms in acute dysenteric stools.

As no vegetative amoebae were discovered in the stools at the time of inoculation, it appeared that those present in such large numbers in the culture the following day, were derived from the cysts. Numerous similar experiments with the freshly-passed faeces of the same patient, and also with those stored at laboratory temperature for three or four days, gave comparable results, as did, likewise, experiments performed with the stools of six or seven other patients which contained the cysts of *Entamoeba histolytica*.

We can therefore accept as a fact that *Entamoeba histolytica* readily excysts on L.E.S. (Locke-egg-serum) medium at 37° C. ; and, as in 24 hours a plentiful growth of vegetative forms can be obtained, the phenomenon can be used with great advantage as a ready means of obtaining a supply of active amoebae.\* For this purpose we found the following procedure to be well-adapted:—

(i) A mass of faeces about the size of a walnut is ground up in a small mortar with a little water, and the emulsion thus formed thoroughly shaken with 500 c.c. of water, and then poured into a tall glass cylinder and allowed to stand for about fifteen minutes. This period is sufficient to permit all the larger and heavier faecal particles to fall to the bottom of the cylinder, and a small scum consisting of very light matter to accumulate at the surface: the scum is removed, and the bulk of the fluid then withdrawn by means of a syphon, leaving only the bottom inch or so and the precipitated mass, which are thrown away. The fluid which has been decanted is now placed in another tall cylinder and allowed to stand over-night, by which time all the cysts, with a certain amount of faecal material, will have settled to the bottom of the cylinder.

The supernatant fluid is again withdrawn, by means of a syphon, and rejected, and the precipitate containing the cysts washed several times by shaking up with water and centrifuging. By this means a deposit is finally obtained which consists of only the finest faecal particles, with relatively few bacteria, and the majority of the cysts. If it be desired to hasten the process, the original fluid, which has been decanted off the coarse faecal deposit after standing fifteen minutes, can be centrifuged immediately, instead of being allowed to stand all night, and the deposit washed repeatedly with water as described above.

If the cysts in the original faeces are scanty, or if for any purpose a particularly high concentration of cysts is required, with relatively

\*Whilst this paper was in preparation, an article by St. John (1926) has appeared, in which it is briefly stated that motile *Entamoeba histolytica* were obtained by sowing on L.E.S. medium a 48-hour old specimen of faeces containing a few *Entamoeba histolytica* cysts; a similar result was also got from the same specimen after it had been kept eight days in the ice-chest.

very little faecal material, the following modification of the above procedure has been found to serve most admirably :—

(ii) As before described, the faeces is ground up in a small mortar with water, and the emulsion shaken up with 500 or 1000 c.c. of water, poured into a tall glass cylinder, and allowed to stand for fifteen minutes to get rid of the coarser faecal material. The supernatant fluid is withdrawn and either centrifuged or allowed to stand over-night in a cylinder: the deposit is then shaken up thoroughly with a solution of cane sugar in water, of a sp. gr. of about 1080, and centrifuged at high speed. This procedure results in separation of the vast majority of the cysts from the remaining faecal material, the faeces being precipitated, and the cysts floating in the supernatant fluid, which is withdrawn, diluted with about four times its volume of water, and again centrifuged at high speed; by this means a small deposit is obtained consisting of great numbers of cysts in a relatively minute quantity of faecal material. The deposit is then washed several times with water, to get rid of all traces of sugar and the majority of the remaining bacteria.

Washed concentrated suspensions of cysts prepared by this method were found to be remarkably satisfactory for obtaining cultures of *Entamoeba histolytica*; the relatively few bacteria which such suspensions contained, as compared with the original faeces, enabled the excysting amoebae to become well-established before the growth of bacteria swamped them.

Careful comparison of the results obtained from cultures of suspensions of cysts, prepared by each of the above methods, has failed to reveal any indication that the concentrated sugar solution has a deleterious effect on the cysts.

The culture tubes should not be placed vertically in the incubator, but should lie so that the top of the egg slope is practically horizontal. In our experience the best way of examining them is to shake the tube vigorously, so that the fluid and solid material (bacteria, amoebae, and débris) on top of the egg slope are thoroughly mixed, and then with a pipette to remove about 1 c.c. and centrifuge it in a warm tube for about half-a-minute; most of the supernatant fluid is then withdrawn and the deposit stirred up with the remainder and examined, preferably in a hot microscope chamber, when the amoebae can readily be detected in large numbers. This procedure appears to us to be much more reliable and satisfactory than merely sampling the bottom of the unshaken culture tube with either a pipette or platinum loop. For sub-inoculation we, likewise, shake the culture tube, remove a few drops with a pipette, and inoculate on to a warm fresh medium; the best results are obtained when subculture is performed daily.

### CHANGES OCCURRING IN CYSTS IN CULTURE TUBES

Before discussing the changes in the cysts which take place on incubation at 37° C. on L.E.S. medium, we should note that the cysts passed by different patients, and even by the same patient at different times, vary greatly in appearance, especially in respect of the number of nuclei they contain, and of their chromatoid bodies and glycogen content. For example, on one occasion the cysts may be practically all uninucleate with much glycogen, and but few of them containing chromatoid bodies, whereas on another occasion the vast majority may be quadrinucleate with chromatoid bodies and but little glycogen. We shall, however, return to this subject in a later communication and here confine ourselves to the changes occurring in the culture tubes.

*Multiplication of nuclei.* Undoubtedly the most striking change is the rapid multiplication of the nuclei in the uni- and bi-nucleate cysts, so that within a few hours practically all the cysts have become quadrinucleate. This is well illustrated in an experiment, details of which are given in Table I. A washed concentrated suspension of *E. histolytica* cysts was quickly made from the freshly-passed faeces of a patient, and inoculated on L.E.S. medium. The cysts in the original suspension, and in samples of the culture taken at short intervals, were carefully examined in iodine and the percentage of uni-, bi-, and quadri-nucleates ascertained. It should be here noted that although a few trinucleate cysts were regularly found, we have not thought it necessary to classify them separately, and in this work they have always been grouped with the quadri-nucleates.

TABLE I.

Showing the conversion of uni- and bi-nucleate cysts into quadri-nucleate cysts during incubation at 37° C. on L.E.S. medium.

Type of cyst	Original suspension	After incubation at 37° C. on L.E.S. medium			
		2 hours	2½ hours	4½ hours	8 hours
Uninucleate ... ..	42	24	8	8	1
Binucleate ... ..	18	13	9	4	0
Quadrinucleate ... ..	27	55	75	53	65
Nuclei indistinct, or cysts shrunken or granular ... ..	13	8	8	3	11
Vegetative amoebae ... ..	...	...	...	32	23

It will be seen from this table that incubation resulted in a steady decline in the percentage of uninucleate, and a corresponding increase in that of quadrinucleate, cysts: within about  $4\frac{1}{2}$  hours, definite numbers of vegetative amoebae began to appear in the cultures. This rapid development is particularly interesting in view of the generally preconceived notion that the cysts do not develop outside the body; e.g., Dobell (1919), writing of *Entamoeba histolytica* cysts states 'Those containing less than four nuclei never develop to maturity outside the body, and usually die much sooner than the mature cysts. Even cysts with dividing nuclei do not complete their nuclear divisions. Spindle-figures and other stages arrested in division can be seen to remain unchanged within the cysts until degeneration takes place.'

In passing, it might be noted that in an appreciable proportion of cysts, both before and after culture, the nuclei were indefinite and sometimes invisible and the cytoplasm appeared granular; in short, the cysts conveyed the impression that they were dead or degenerating.

We have been unable to discover any evidence that, during the development of the cysts, autogamy, such as has been described by Wenyon (1907), in the case of *Entamoeba muris*, occurs. We have observed in the living cysts the multiplication of the nucleus with the formation of four daughter nuclei, the agglomeration of the nuclei, and the excystment of the 4-nucleated amoeba. We have seen nothing suggestive of fusion of the daughter nuclei either in living or stained preparations. In a recent letter, Dr. Wenyon informs us that he now believes he was mistaken regarding the occurrence of autogamy in the cysts of *Entamoeba muris*, and that he is stating so in his forthcoming book on Protozoology.

On various occasions, what appeared to be particles of chromatin were seen to be extruded from the nucleus during development of the cysts. Such appearances are illustrated in Pl. XXI, figs. 3 and 4, and it seems probable that a reduction of chromatin is associated with nuclear division.

So far as we have been able to ascertain, the following changes occur in nuclear division. The karyosome appears to lose its definite outline and to become fragmented; this is possibly associated with extrusion of chromatin particles from the nucleus (Pl. XXI, fig. 3);

two daughter karyosomes then make their appearance, and the nucleus elongates in a spindle-shaped manner and appears to be traversed in a longitudinal direction by numerous fibrils arranged irregularly (Pl. XXI, figs. 4 to 7); a constriction then appears about the equator and the ends become rounded, the nucleus assuming a dumb-bell-shaped appearance; finally, the neck constricts and division is completed (Pl. XXI, figs. 8 to 10).

*Glycogen content.* Another very definite change seen in the cysts during culture was the rapid disappearance of glycogen. When first passed practically all the uninucleate cysts contained a more or less large mass of glycogen which stained deeply with iodine, and to some extent frequently masked the nucleus: this was seen also in the binucleate cysts, but to a much less extent; the quadri-nucleate cysts rarely contained any appreciable quantity of glycogen, and as a result stained much less deeply than the uninucleate cysts, and the nuclei were clearly visible.

*Chromatoid bodies.* Careful examination of freshly-passed faeces showed that these bodies were comparatively rarely present in the uninucleate cysts, but were more commonly found in the bi- and quadri-nucleates. Study of cultures indicates that there is a definite cycle in their development; they were found in a much larger proportion of cysts after a few hours' incubation than before, but finally, in still older cultures, in which vegetative amoebae were beginning to appear, they were greatly diminished in number, and there is reason to believe that in the vast majority of cysts the chromatoid bodies disappear before excystation.

The changes during culture undergone by the cysts in respect of the number of nuclei, glycogen content, and chromatoid bodies, are well shown in an experiment, details of which are set forth in Table II.

This table shows clearly the development of the nuclei during culture, the quadrinucleates which in the original faeces comprised 40 per cent. of the total cysts, after  $5\frac{1}{2}$  hours' incubation on L.E.S. medium at  $37^{\circ}$  C. had (including the quadrinucleate amoebae) increased to 88 per cent.; the progressive disappearance of glycogen, and the initial increase, and subsequent decrease, in the percentage of cysts containing chromatoid bodies are equally apparent.



TABLE II.

Showing the changes undergone by *Entamoeba histolytica* cysts during cultivation on L.E.S. medium at 37° C., in respect of the number of nuclei, glycogen content, and chromatoid bodies.

No. of hours incubated		Number of nuclei			Nuclei indistinct, or cysts shrunken or granular	Vegetative amoebae
		1	2	4		
Original faeces ...	Glycogen only ...	21	9	7	...	...
	Chromatoids only ...	1	2	6	...	...
	Glycogen + chromatoids	4	6	8	...	...
	Neither ...	8	8	19	...	...
	Total ...	34	25	40	1	0
1½ hours ...	Glycogen only ...	7	3	3	...	...
	Chromatoids only ...	6	6	33	...	...
	Glycogen + chromatoids	3	2	4	...	...
	Neither ...	9	4	16	...	...
	Total ...	25	15	56	4	0
3½ hours ...	Glycogen only ...	0	0	0	...	...
	Chromatoids only ...	5	12	39	...	...
	Glycogen + chromatoids	0	0	0	...	...
	Neither ...	5	3	23	...	...
	Total ...	10	15	62	9	4*
5½ hours ...	Glycogen only ...	0	0	0	...	...
	Chromatoids only ...	1	1	10	...	...
	Glycogen + chromatoids	0	0	0	...	...
	Neither ...	3	2	49	...	...
	Total ...	4	3	59	5	29*

\* All contained four nuclei and none contained either glycogen or chromatoid bodies.

### EXCYSTMENT OF *ENTAMOEBA HISTOLYTICA* IN CULTURES

On many occasions amoebae were actually observed to excyst : as already pointed out, the preparatory stages leading up to this appear to be multiplication of the nucleus and agglomeration of the four daughter nuclei, disappearance of the glycogen mass, and the formation and final disappearance of the chromatoid bodies. An individual which is about to excyst presents a characteristic appearance. The cytoplasm is more or less homogeneous and appears to be of a faintly-greenish tint, and is frequently very finely-alveolar ; the nuclei in the living individual can be distinguished only with the

greatest difficulty. Careful examination shows that the amoeba is retracted in places from the cyst envelope and is evidently loose inside it; from time to time vigorous pseudopodial movements can be seen to take place (Pl. XXII, figs. 1 to 8). Finally, a rent apparently occurs in the cyst envelope, and a clear bead of ectoplasm is protruded; this progressively enlarges in a spasmodic manner, more and more of the amoeba protruding from the envelope, until finally the creature has escaped completely (Pl. XXII, figs. 9 to 15). It then proceeds to move about in an active, usually slug-like manner, frequently drawing behind it the empty cyst envelope or faecal débris. In the actively moving freshly-excysted *Entamoeba histolytica* the agglomerated nuclei are almost invariably to be found in the anterior part of the creature, and can easily be seen streaming into the pseudopodium immediately after this is protruded; the hindermost portion of the amoeba appears to take little part in active movement and seems to be dragged behind the advancing parasite as a sort of tail, and it is to this that the cyst envelope, or faecal débris, is often adherent (Pl. XXII, figs. 16 to 18). Although at the moment of emergence the cytoplasm is either practically homogeneous or, at most, very finely alveolar, with minute granules, it quickly becomes definitely alveolar, and as it ingests bacteria digestive vacuoles appear in large numbers.

For the study of living examples we employed the following method:—

The upper surface of a warmed microscope slide was coated with melted agar which was then allowed to solidify. The slide was then warmed to 37° C. and a drop of the material to be observed placed on it and covered with a slip, the under surface of which had been coated with agar in a similar manner; the preparation was carefully sealed with paraffin in order to prevent evaporation, and observed in the warm microscope chamber. Actual excystation can be better observed by mounting in this way material from a two-hour old culture of cysts on I.E.S. medium, than by using a suspension in Locke-serum of previously unincubated cysts.

The cyst envelope, after the escape of the amoeba, usually appears as a complete sphere with an extremely delicate wall; the rent through which the amoeba has emerged is very often invisible, but at times, by careful focussing and suitable illumination, it can be clearly seen (Pl. XXII, fig. 11). How long the cyst envelopes remain as such we cannot say, but we have found them in considerable numbers, frequently full of bacteria, in cultures twenty-four hours old.

## DEVELOPMENT OF THE EXCYSTED AMOEBÆ

Our observations confirm, then, those of Penfold, Woodcock, and Drew, and of Chatton, that freshly-excysted *Entamoeba histolytica* are quadrinucleate, and suggest that Darling was mistaken when he described the division of the 4-nucleated encysted amoeba into four small amoebulae before excystment. As Chatton has pointed out, the nuclei of the quadrinucleate individuals are agglomerated together in a very characteristic manner. We cannot agree with Dobell (1919) that the nuclear agglomeration is, in this case, any indication of degeneration; on the contrary, we believe it to be an absolutely constant and normal phenomenon. The subsequent development of the quadrinucleate amoebae is a matter of considerable interest; at first we believed that they all quickly divided into uninucleate organisms, because these were found in large numbers in slightly older cultures. On examining, however, a fresh preparation of a culture about 16 hours old, we were much impressed with the relatively enormous size of a number of the amoebae, some of them, in fact, were of such gigantic dimensions that they occupied practically the entire microscope field ( $\frac{1}{12}$  Obj., 4 Oc.). These individuals, although exhibiting considerable pseudopodial movement, did not, as a rule, show the fairly rapid translatory motion so characteristic of the ordinary amoebae. On examining them carefully it was seen that they were multinucleate, and on running iodine under the coverslip it was found that sometimes as many as 30 to 40, or occasionally even more, nuclei could be counted. These gigantic amoebae were discovered only in cultures of from about 10 to 30 hours old, and only in those originating from the inoculation of cysts (Pl. XXIV, figs. 1 to 6). We have never seen them in subcultures, or in cultures originating from the inoculation of vegetative amoebae from acute dysenteric stools. Consequently, it appears only reasonable to conclude that they are derived from the quadrinucleate excysted individuals, the nuclei of which continue to divide in the normal manner without a corresponding division of the cytoplasm. The proportion which these multinucleate individuals formed, varied considerably in different cultures; generally they appeared to comprise but a comparatively insignificant percentage, but in some preparations they constituted as much as 20 to 24 per cent. of the total amoebae.

With a view to investigating the matter more closely, we performed a number of experiments which consisted in ascertaining, at stated intervals, the percentage of the various types of cyst and vegetative forms present in a culture, on L.E.S. medium, of a suspension of washed cysts; the preparations were stained with iodine. In these experiments, in order to avoid disturbing the cultures by frequent sampling, we inoculated a series of tubes so that each was examined on a single occasion only, and then discarded. As the experiment, which was repeated many times, on cultures made from cysts obtained from a number of different patients, always gave similar results, it will suffice if we consider but a single example, details of which are set forth in Table III.

TABLE III.

Showing the percentage of different types of cysts and of vegetative individuals, in cultures of various ages, made by sowing, on L.E.S. medium, cysts from a chronic dysenteric patient.

No. of hours incubated	Cysts					Vegetative amoebae				
	No. of nuclei			Nuclei indistinct, or cysts shrunk or granular	Cyst envelopes	No. of nuclei				Multi- nucleates
	1	2	4			1	2	3	4	
Original suspensions	57	18	15	10	...	...	...	...	...	...
2 hours ...	23	14	30	33	...	...	...	...	...	...
3½ hours ...	4	8	41	33	2	0	2	0	12	0
6½ hours ...	8	5	37	25	2	0	5	1	19	0
10½ hours	0	1	1	8	1	31	17	5	36	1
13 hours ...	1	0	3	10	2	48	18	5	12	3
16 hours ...	1	0	0	3	...	49	22	8	5	12
19 hours ...	1	0	2	3	...	63	15	6	2	8
24 hours ...	...	...	...	2	1	74	14	1	3	6
30 hours ...	...	...	1	1	...	86	5	0	2	5
33 hours ...	...	...	...	...	...	84	6	2	2	6
36 hours ...	...	...	...	...	...	90	5	2	0	3
48 hours ...	...	...	...	...	...	97	3	0	0	0

The developmental changes, which are disclosed in this table, are quite definite, and can be regarded as typical of what occurs in any L.E.S. culture of *Entamoeba histolytica* cysts. It will be observed that, in this particular case, the suspension of washed cysts, made from freshly-passed faeces, contained a marked preponderance of uninucleates: after 2 hours the cysts had so developed that the quadrinucleate individuals outnumbered the uninucleate. In  $3\frac{1}{2}$  hours the uninucleates had decreased enormously in number and formed only a small fraction of the total cysts: at this period there was definite evidence of excystation, 14 per cent. of the individuals seen being vegetative forms. The  $6\frac{1}{2}$ -hour culture presented much the same appearance, except that the number of vegetative forms had increased to 25 per cent. of the total. These last two cultures afford conclusive proof that the freshly-excysted individuals are quadrinucleate; no uninucleate forms were seen, and only comparatively few bi- and tri-nucleate individuals.

It will be observed that in the 2-hour, the  $3\frac{1}{2}$ -hour, and the  $6\frac{1}{2}$ -hour cultures there was a considerable increase of individuals falling into the 'Nuclei indistinct, or cysts shrunken or granular' category; undoubtedly some of these were dead or degenerate, but certainly many were forms in the stage immediately prior to excystment. As we have already pointed out, the contents of a cyst shortly before excystation become amoeboid and slightly withdrawn from the cyst envelope, and the nuclei are closely agglomerated. When such forms are stained by running iodine into the preparation, the shrinkage is often accentuated and the nuclear agglomeration, although it can be seen, becomes obscured and difficult to resolve into its four constituents. That this interpretation is correct is shown by the fact that in cultures over 6 hours old the number of individuals falling into the category in question had returned to about that existing in the original suspension. It must be admitted that in iodine-stained preparations of early cultures it is by no means easy to decide in all cases whether an individual is on the point of excysting, or whether it is dead and degenerate; but it can be laid down as a general rule that in pre-excysting forms the cytoplasm is slightly alveolar and at most very finely granular, and the nuclear agglomeration can always be seen, although it is often somewhat indefinite and not clearly resolvable into its four constituents.

(Plate XXI, figs. 19 and 20); whilst in the dead and degenerate forms the cytoplasm is much more coarsely granular and the nuclei are either distinct, and if more than one is present, separate, or else in advanced stages invisible; in such cases shrinkage of the contents from the envelope is often very pronounced (Plate XXI, figs. 21 to 24). In the 10½-hour old culture practically all the cysts have disappeared, except for a number obviously dead and degenerate; almost 90 per cent. of the individuals are vegetative forms, and whilst the quadrinucleate amoebae are still the most numerous, a relatively large number of uni-, and bi-nucleates are present, as well as a very few multinucleate (i.e., having more than four nuclei) forms. There is thus at this point evidence that some of the quadrinucleate amoebae are dividing into tri-, and bi-, and uni-nucleate individuals, and that in a few of them the nuclei are multiplying without division of the cytoplasm. In the next two cultures, 13 and 16 hours old, respectively, the decrease in number of the quadrinucleate amoebae, with a corresponding increase of uni-nucleates and multinucleates, is clearly seen. In still older cultures—19 to 48 hours—the proportion of uninucleate individuals steadily increases, whilst that of the multinucleates gradually dwindles to nothing.

Yoshida (1920) has described in freshly-excysted *Entamoeba tetragena*, and also in *Entamoeba coli*, fusion of the nuclei: this is stated to be of two types, the first a simple autogamy involving the fusion of two nuclei, and the second a polynuclear autogamy involving the fusion of several nuclei with the formation of a syncaryon. We have never been able to observe anything of this nature, either in fresh or in stained preparations. We believe the usual fate of the excysted quadrinucleate *Entamoeba histolytica* is its subdivision into four uninucleate amoebae, either by first dividing into two binucleate individuals, each of which again subdivides into two uninucleates, or probably most commonly by throwing off uninucleates one at a time. Plate XXIII, figs. 2 to 5 are camera lucida drawings of individuals from cultures 11 to 17 hours old, that is at a time when the quadrinucleate excysted amoebae were becoming converted into uninucleate individuals, and show the separation of one or two nuclei from the original agglomeration, a process which we believe to precede division of the quadrinucleate parasites.

### UNDER WHAT CONDITIONS DOES *ENTAMOEBA HISTOLYTICA* EXCYST ?

Having ascertained that *Entamoeba histolytica* rapidly excysts when sown on L.E.S. medium, and incubated at 37° C., and that under such circumstances a growth of vegetative forms is readily obtained, we decided to investigate whether excystation would take place under other conditions. It was found that when nutrient agar, or nasgar, was substituted for the egg medium, and the ordinary mixture of Locke's fluid (eight parts) and serum (one part) added, results exactly similar to those obtained on L.E.S. medium were observed ; the cysts developed rapidly, excystment occurred, and the quadrinucleate amoebae divided into uninucleate, or formed multinucleate, individuals just as has been described in the experiments where L.E.S. medium was used.

In other experiments about 13 drops of suspension of washed *Entamoeba histolytica* cysts were added to tubes containing respectively 5 c.c. of Locke-serum, 5 c.c. of broth, or 5 c.c. of physiological saline. In all cases motile vegetative amoebae and cyst envelopes were seen after a few hours' incubation at 37° C. ; furthermore, in each of the above fluids actual excystment was observed. The cysts themselves gradually disappeared, and after 24 to 36 hours nothing but a few obviously degenerate cysts was to be seen. Although the excysted amoebae could be observed to be moving actively for a time, they failed to multiply as on the L.E.S., Agar L.S., or Nasgar L.S. media, and soon died.

When 13 drops of the suspension of washed cysts were added to 5 c.c. of water and incubated at 37° C., definite changes occurred, which, so far as they went, were quite comparable with those recorded above. The majority of the uninucleate cysts became quadrinucleate just as on any of the nutrient media. No active vegetative amoebae were found, but occasional cyst envelopes were seen and sometimes the initial stages of excystment. Our observations left us in no doubt that, in many cysts, the normal development continued so far as rupture of the cyst-envelope : when this happened the amoebae were quickly killed by the water ; as a rule the creature died before escaping from the envelope, sometimes it died when partly excysted, and sometimes it actually escaped from the envelope before perishing.

It seems quite clear that the suspension of the cysts in water at 37° C. is not deleterious to the cysts, but only to the excysting amoebae; subcultures made on L.E.S. medium, from the water suspension after 4 or 6 hours' incubation, resulted in a good growth of vegetative amoebae.

So far as we have been able to ascertain the conditions essential for the development and excystation of *Entamoeba histolytica* are moisture and a suitable temperature, preferably about 37° C.

### SUMMARY

1. *Entamoeba histolytica* cysts develop and excyst under suitable conditions *in vitro*.

2. So far as we have been able to ascertain, moisture and a suitable temperature (preferably about 37° C.) are essential for the occurrence of excystation; the passage of the cysts through such solutions as *liquor pepticus* or *liquor pancreaticus* is unnecessary for excystation.

3. The changes attending development and excystation can readily be followed in cultures of *Entamoeba histolytica* cysts on L.E.S. and certain other media at 37° C.

4. Great variation in the stage of development of *Entamoeba histolytica* cysts may be seen in the freshly-passed faeces of different individuals, and of the same individual at different times. These variations concern chiefly the number of nuclei, the glycogen content, and the percentage containing chromatoid bodies.

5. The youngest cysts are uninucleate and loaded with glycogen; as development takes place chromatoid bodies make their appearance, the nuclei divide, and the glycogen decreases in amount. Later, the cyst becomes quadrinucleate; chromatoid bodies are well-developed, but the glycogen is much less evident, or entirely absent. When the cyst is completely mature, and ready to excyst, the nuclei are agglomerated and the cytoplasm is homogeneous and without glycogen; the chromatoid bodies are greatly reduced or absent.

6. We have failed to observe any evidence of autogamy in the development of the cysts, but we have frequently seen chromatin particles apparently extruded from the nuclei immediately prior to, or during, division.



7. The freshly-excysted *Entamoeba histolytica* contains four closely agglomerated nuclei, and the cytoplasm is very finely-alveolar. It moves in a characteristic slug-like manner, with the nuclei almost invariably anterior, and drags behind a more or less motionless tail; as bacteria are ingested the cytoplasm becomes more alveolar and digestive vacuoles quickly appear.

8. In cultures the majority of the quadrinucleate excysted amoebae divide either directly, or indirectly, into four uninucleate individuals, but in a certain proportion the cytoplasm fails to divide and multinucleate individuals are formed. Here again we were unable to observe any indication of autogamy.

9. A method of obtaining concentrated washed suspensions of *Entamoeba histolytica* cysts is described. Inoculation on L.E.S., or other suitable, medium with such suspensions is the simplest way of obtaining excellent cultures of vegetative forms.

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## EXPLANATION OF PLATE XXI

All figures are camera lucida drawings of *Entamoeba histolytica* cysts stained with iodine ; magnification about 1,730. The figures represent the various stages in the development of the cysts as seen either in freshly-passed faeces or in cultures.

Fig. 1. Uninucleate cyst with a large glycogen mass.

Fig. 2. Uninucleate cyst with a glycogen mass and a chromatoid body.

Figs. 3 and 4. Uninucleate cysts showing fragmentation of the karyosome and extrusion of chromatin particles.

Figs. 5 to 8. Various stages in the division of the nucleus in uninucleate cysts.

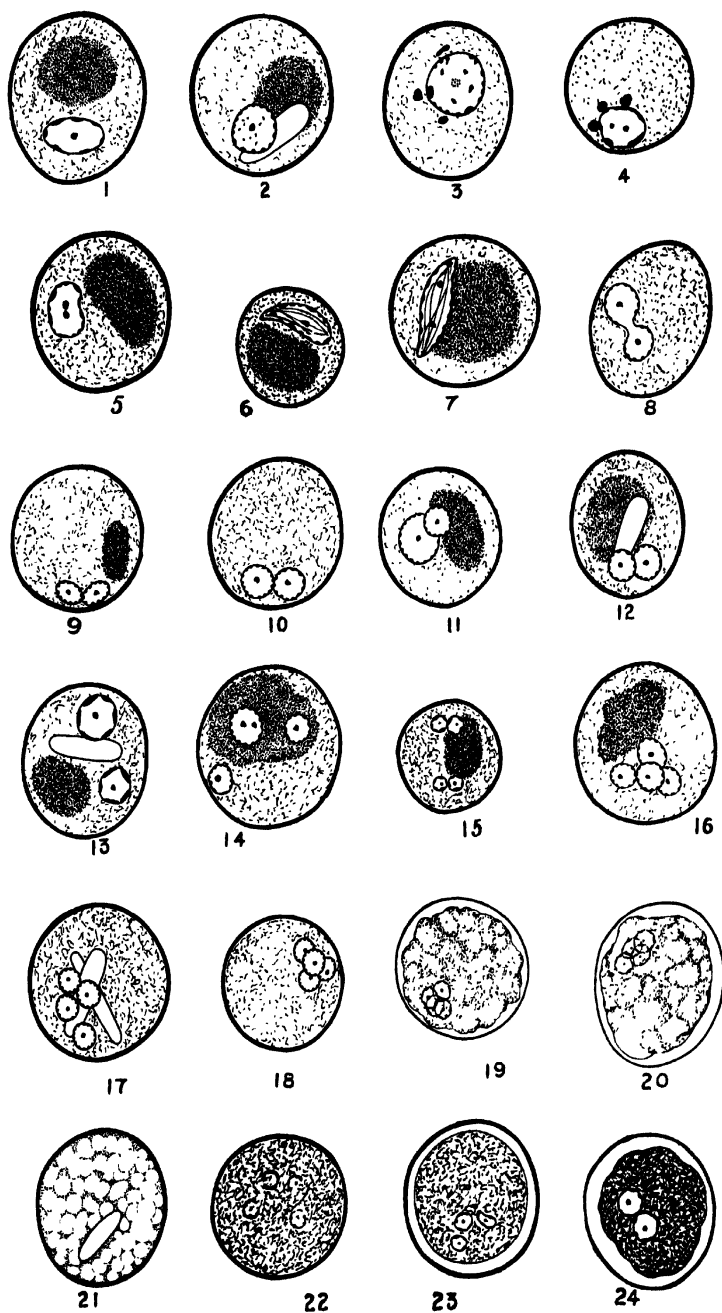
Figs. 9 to 13. Various types of binucleate cysts.

Fig. 14. Trinucleate cyst.

Figs. 15 to 18. Various types of quadrinucleate cysts.

Figs. 19 and 20. Pre-excysting forms: these individuals were actually seen to be moving within the cyst-envelope before iodine was run into the preparation.

Figs. 21 to 24. Various dead and degenerate cysts.







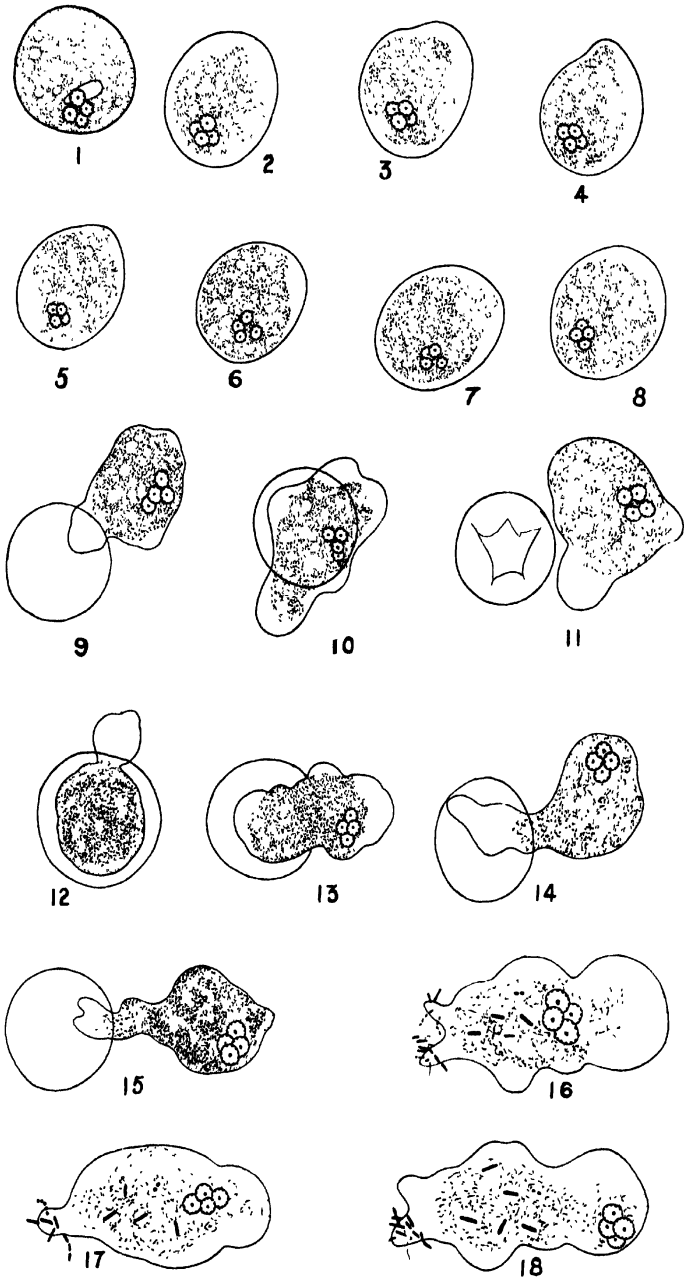
## EXPLANATION OF PLATE XXII

All figures are camera lucida drawings of living specimens of *Entamoeba histolytica* : magnification about 1,500.

Figs. 1 to 11. Showing the successive changes occurring in a single cyst kept at 37° C. and observed over a period of about an hour ; Fig. 1, a mature 4-nucleated cyst with the remnant of the chromatoid body and agglomeration of the nuclei ; Figs. 2 to 8, showing the total disappearance of the chromatoid body, the retraction of the cytoplasm from the cyst envelope, and the amoeboid movements of the parasite within the envelope ; Figs. 9 to 11, the escape of the amoeba from the cyst envelope.

Figs. 12 to 15. Various stages in excystation observed in the case of another cyst.

Figs. 16 to 18. A recently excysted amoeba showing the agglomerated nuclei following closely the advancing pseudopodium, and the more or less motionless 'tail' to which are attached bacteria and débris.









## EXPLANATION OF PLATE XXIII

All figures are camera lucida drawings of iodine-stained preparations of *Entamoeba histolytica* as seen in 10 to 20 hour-old cultures of cysts. Magnification about 1,500.

The figures show what we believe to represent the usual fate of the quadrinucleate excysted amoebae, viz., their division into tri-, bi-, and finally, uni-nucleate individuals.

Fig. 1. Typical quadrinucleate amoeba showing nuclear agglomeration.

Figs. 2 to 4. Amoeba showing separation of one nucleus from the agglomeration, presumably preparatory to the separation from the parent of a uninucleate individual.

Fig. 5. Showing simultaneous separation of two of the nuclei from the agglomeration.

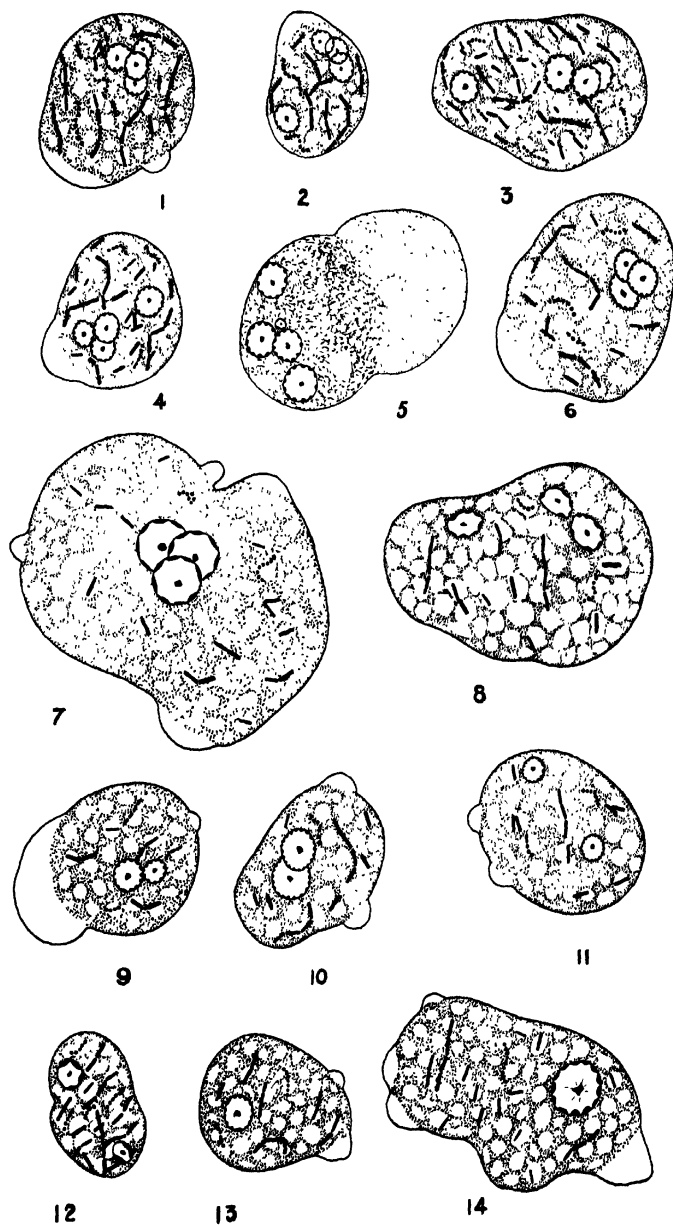
Figs. 6 and 7. Trinucleate amoebae with agglomerated nuclei.

Fig. 8. Separation of one of the nuclei in a trinucleate amoeba.

Figs. 9 and 10. Binucleate amoebae with agglomerated nuclei.

Figs. 11 and 12. Separation of the nuclei of binucleate amoebae preparatory to division.

Figs. 13 and 14. Uninucleate amoebae.



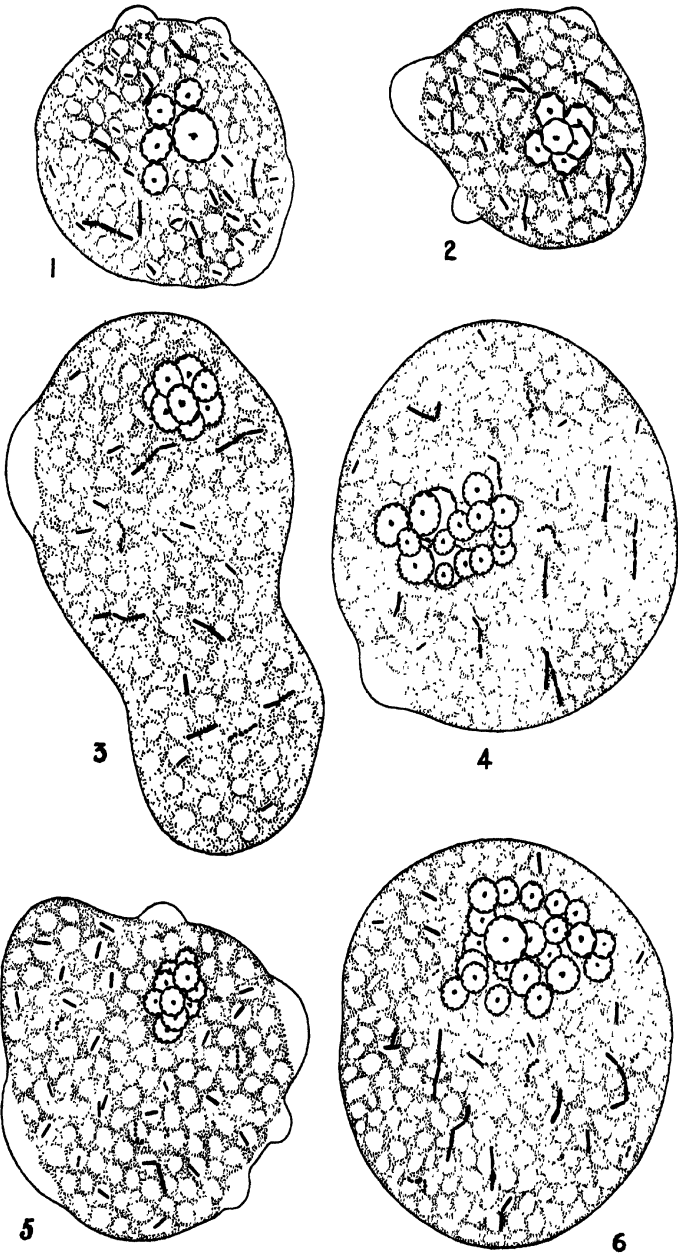




## EXPLANATION OF PLATE XXIV

All figures are camera lucida drawings of iodine-stained preparations of *Entamoeba histolytica* as seen in 10 to 20 hour-old cultures of cysts. Magnification about 1,500.

Figs. 1 to 6. Typical examples of multinucleate amoebae.







# A COMPARATIVE STUDY OF SODIUM CITRATE AND TARTRATE IN THE DIFFERENTIATION OF *B. COLI* IN WATER ANALYSIS

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PLATES XXV-XXVII

Brown (1921), in a study of the use of citrated media for the cultivation of micro-organisms, showed that in a 1 per cent. sodium citrate broth certain bacteria fail to grow, and the addition to the cultures after forty-eight hours' incubation, of a saturated solution of lead acetate, produces a copious white precipitate, whilst other organisms utilise the citrate for growth and lead acetate yields only a slight precipitate.

Stewart Koser (1923), in an exhaustive study of the utilisation of salts of various organic acids by the colon aerogenes group of organisms, showed that the group is divisible into two sections, one a *B. coli*, or faecal type, which fails to utilise sodium citrate in a synthetic medium, and the other a *B. aerogenes*, or non-faecal type, which produces a visible turbidity, usually within forty-eight hours.

Brown, Duncan, and Henry (1923), in an extensive study of the fermentation of salts of organic acids as an aid to the differentiation of bacterial types, showed that with stock strains of organisms *B. lactis aerogenes* and *B. cloacae* are capable of decomposing citrate, while *B. coli communis*, *B. coli communior* and *B. acidi lactici* fail to attack citrate, and that this difference is also recognisable by the use of lead acetate in a definite quantitative relation to the medium employed.

In previous communications the results of the application of Koser's citrate medium to colon organisms isolated in Trinidad, from faeces, unpolluted soils and waters of various degrees of sanitary purity were recorded, and the suggestion made that in the bacterio-

logical analysis of unpurified and doubtful water supplies it should be employed as a means of differentiation between faecal and non-faecal coli organisms.

In the present contribution will be seen the findings of a comparative study of the results obtained with Koser's medium, and the methods recommended by Brown, Duncan, and Henry, as applied to coli organisms isolated directly from faeces and from water supplies in Trinidad.

Koser's medium was the one employed in previous studies and already described. It consisted of 1.5 grammes microcosmic salt,  $\text{Na}(\text{NH}_4)\text{HPO}_4$ , 4 aq., 1 gramme  $\text{KH}_2\text{PO}_4$ , 0.2 gramme  $\text{MgSO}_4$ , and 2 grammes sod. citrate in 1,000 c.c. distilled water, tubed and autoclaved at  $120^\circ\text{C}$ . for fifteen minutes. The cultures were incubated at  $30^\circ\text{C}$ . for two to three days, after which time readings were made, a clear colourless liquid indicating a faecal organism, and turbidity with a slight deposit, a non-faecal one. The medium recommended by Brown, Duncan, and Henry, consisted of 1 per cent. sod. citrate, or 1 per cent. sod. tartrate in a 1 per cent. peptone water (bacto-peptone of the Digestive Ferment Co., 1 gramme, sod. chloride 0.05 gramme, aq. distil. 100 c.c.). About 3 c.c. portions were tubed and autoclaved at  $115^\circ\text{C}$ . for twenty minutes. The cultures were incubated at  $37^\circ\text{C}$ . for forty-eight hours, and three drops of a saturated solution of lead acetate added, the tubes well shaken and examined the following day. In one series of experiments the lead acetate was added to the culture tubes in the definite proportions recommended by Brown and his associates. The formation of a voluminous white flocculent precipitate was evidence that the salt was not decomposed and the organism was of faecal origin. Should a small granular precipitate form which slowly settles to the bottom of the tube, decomposition of the salt was considered to have taken place and the organism to have been a non-faecal one.

Fifty, *B. coli* colonies (lactose + indol +) were isolated from five different samples of cow dung secured from five different bovines. All these fifty colonies failed to produce turbidity in Koser's medium, in accordance with their faecal origin, except colony No. 21, which showed a faint turbidity *without deposit* in three days. In the tartrate medium all fifty colonies produced a heavy white flocculent precipitate, agreeing with their faecal source.

In a similar manner fifty colon organisms were isolated from three different samples of human faeces from three different individuals. All these colonies fermented lactose with the production of acid and gas, and yielded indol by Ehrlich's method. All failed to utilise the citrate and tartrate in the bacto-peptone media and the addition of lead acetate produced an abundant white flocculent precipitate as would be expected of faecal coli. In Koser's citrate media they also failed to produce turbidity, agreeing with the characteristic of faecal colon organisms, with the exception of one colony which produced a visible turbidity *but no deposit* in seventy-two hours. As in the previous experiments there was no quantitative relation between the lead acetate and the bacto-peptone media in the performance of these tests.

These results, though obtained with a small number of cultures, indicate that these media and methods may be of distinct value in detecting the presence of colon organisms isolated from human faeces and cow-dung, but it was necessary to submit them to the action of colon organisms isolated from water supplies to justify an expression of opinion as to their comparative value in water bacteriology.

Two samples (25 c.c. each) of water were collected from an undeniably pure source, viz., the Chorah Ravine. This stream rises from an uninhabited and wooded land, free from all possibility of human pollution and without any evidence of animal faecal contamination, to flow subterraneously for about fifty yards and escape at the side of a hillock. From this outlet the samples were taken. Forty colon colonies were obtained in the routine way and inoculation made into lactose-peptone water, Witte's peptone water, Koser citrate and citrate and tartrate bacto-peptone, and tested as above explained. All the forty colonies fermented lactose with the production of acidity and gas, all produced indol except colonies No. 7 and No. 15, and they all utilised Koser's citrate with the production of a visible turbidity indicative of a non-faecal origin. In the citrate bacto-peptone medium with the addition of lead acetate, only seventeen were found to yield a slight granular precipitate, which is considered to be evidence of a non-faecal origin, and the remaining twenty-three produced a heavy white flocculent precipitate indicative of a faecal source. Both the colonies which

failed to produce indol (i.e., Nos. 7 and 15) yielded in citrate bacto-peptone a slight granular precipitate. In the tartrate bacto-peptone all the forty colonies produced a heavy white precipitate characteristic of faecal coli.

It is thus seen that, of the three methods, Koser's correlates most accurately with the origin of the cultures when obtained from a sanitariously pure source.

Table I gives these various results.

TABLE I.

Koser's citrate		Bacto-peptone citrate		Bacto-peptone tartrate		No. of Colonies	Source
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.		
1 ?	49	—	—	0	50	50	Cow Dung
1 ?	49	0	50	0	50	50	Human Faeces
40	0	17	23	0	40	40	Unpolluted Water

Positive = utilisation of citrate = non-faecal organism.

Negative = no utilisation of citrate = faecal organism.

It should be noted that the lead acetate was not added in measured proportion to the media:

Whilst, therefore, of one hundred colon organisms isolated from faeces, 98 per cent. were definitely faecal in their action upon Koser's citrate, and 2 per cent. may be considered doubtful; of fifty such organisms isolated from cow-dung, and fifty from human faeces, 100 per cent. were faecal in their mode of action upon bacto-peptone tartrate and citrate. On the other hand, of forty colon organisms obtained from an undeniably pure water supply, whilst 100 per cent. were non-faecal in their action upon Koser's citrate, only 42·5 per cent. can be considered as of non-faecal origin in the case of bacto-peptone citrate, and with bacto-peptone tartrate they would all be regarded as having a faecal source and the water as being grossly contaminated. Here again it cannot be too strongly emphasised that the valuation of any reaction supposedly characteristic of *B. coli* should be based upon an examination of *B. coli* as isolated not only from faeces but also from waters of various degrees of sanitary purity.

In addition, forty coli-like colonies were isolated in the height of the dry season from four different samples (25 c.c. each) of water collected from the Maraval River below the Maraval Reservoir. At the Reservoir the river water is chlorinated so as to remove lactose fermenters from 50 c.c. On 29 March, with the cleaning of the tanks, this chlorinated water was allowed to escape into the river which flows along a public road exposed to pollution from a few houses in the neighbourhood and which also receives the morning overflow. The samples were collected on 29 March, about one mile below the Reservoir. These forty colonies, all of which were lactose fermenters, and thirty-nine of which were indol producers, were subjected to the action of Koser's citrate, bacto-peptone citrate and tartrate in the manner already described, with the following results.

TABLE II.

Lactose		Indol		Koser's citrate		Bacto-peptone citrate		Bacto-peptone tartrate	
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
40	0	39	1	21	19	18	22	13	27

In order, however, to correlate more closely the results of sanitary and topographical survey with the bacteriological findings, samples of water were collected from the Lopinot River at various levels, as shown on the sketch map, by skirting the bank of the river in a motor car, and 25 c.c. from each sample were inoculated into MacConkey lactose bile salt medium, double strength, plates being made on Rebipelagar and red or reddish colonies picked out on to agar slants on which they were kept for some months until ready for use, when a further subculture on agar was made and growth allowed to proceed for twenty-four hours. To follow strictly the procedure recommended by Brown and his associates, from the agar slants, inoculations were made into peptone broth and allowed to incubate for twenty-four hours at 37.5° C. From the broth tubes about 3 mm. loopfuls were added to citrate bacto-peptone water, tartrate bacto-

peptone water, Koser's citrate, Witte's peptone water and lactose-peptone water. For the sake of economy the bacto-peptone media were tubed in 2.5 c.c. quantities and after twenty-four hours' incubation at 37.5° C., 0.2 c.c. of an aqueous saturated solution of lead acetate was added to the citrate and 0.3 c.c. to the tartrate, in the proportion recommended by Brown and his co-workers. Koser's citrate tubes were read in three days' time after incubation at 30° C., and Ehrlich's Para-dimethyl-amido-benzaldehyde test for indol performed after three days' incubation at 37° C. The results are shown on sketch map (p. 311), and in Table III.

The lack of correlation between the bacteriological findings and the sanitary survey in the case of the tartrate medium, as exemplified by the results obtained with both the Chorah and Lopinot waters, particularly with the former, indicates that this medium conveys little or no information as to the degree of purity of a water supply and may be disregarded in routine bacteriological analysis. In connection with the bacto-peptone citrate and Koser's citrate, the results from the Maraval and Lopinot Rivers show that the difference between these media is small, but when the Chorah water results are examined it is seen that Koser's citrate reveals more accurately the source of the cultures.

It should, however, be remembered that it may not be possible with Koser's citrate to express an opinion as to the sanitary source of a colon organism until incubation has proceeded for more than two to three days, and there may still be an element of doubt, whilst the application of the bacto-peptone citrate medium necessitates the additional use of another reagent and more labour.

Photographs 1 to 9, illustrating the nature of the precipitate obtained with the bacto-peptone media, explain themselves. It is seen that the precipitate formed when the lead acetate solution is not added in the definite measured proportion recommended by Brown and his associates may vary considerably in quantity.

### TABLE III.

Showing results of the action of coli organisms isolated from various levels of the Lopinot River  
Trinidad, upon sodium citrate and tartrate.

	Lactose	Indol	Koser's citrate	Bacto- peptone citrate	Bacto- peptone tartrate	
1	+	-	+	+	+	Sample taken at 8½ miles. Upper part of River. <i>Little or no evidence of Pollution.</i>
2	+	-	+	+	-	
3	+	+	+	+	-	
4	+	+	+	+	-	
5	+	+	+	+	-	
6	+	+	+	+	-	
7	+	-	+	+	-	
8	+	-	+	+	-	
9	+	-	-	+	-	
10	+	-	+	+	-	
11	+	-	+	+	+	
12	+	-	+	+	+	
13	+	-	+	+	+	
14	+	-	+	+	-	
15	+	+	+	+	-	
16	+	+	+	+	+	
17	+	-	+	+	+	
18	+	-	+	+	+	
19	+	-	+	-	+	
20	+	-	+	+	+	
21	+	+	+	-	-	Sample taken at 7½ miles. Lower down stream than previous. <i>Some Pollution.</i>
22	+	+	-	-	-	
23	+	+	-	-	-	
24	+	+	+	-	-	
25	+	+	-	-	-	
26	+	+	-	-	-	
27	+	+	-	-	-	
28	+	+	-	-	-	
29	+	+	-	-	-	
30	+	+	-	-	-	
31	+	+	+	-	-	
32	+	+	+	-	+	
33	+	+	+	-	-	
34	+	+	-	-	-	
35	+	+	+	-	+	
36	+	+	-	+	-	
37	+	+	-	-	-	
38	+	+	+	-	-	
39	+	+	+	+	-	
40	+	+	-	-	-	
41	+	+	-	-	-	Sample taken at 7½ miles. <i>Much Pollution.</i>
42	+	+	-	-	-	
43	+	+	-	-	-	
44	+	+	-	-	-	
45	+	+	-	-	-	
46	+	+	-	-	-	
47	+	+	-	-	-	
48	+	+	-	-	-	
49	+	+	-	-	-	
50	+	+	-	-	-	
51	+	+	-	-	-	
52	+	+	-	-	-	
53	+	+	-	-	-	
54	+	+	+	+	-	
55	+	+	-	-	-	
56	+	+	-	-	-	
57	+	+	-	-	-	
58	+	+	-	-	-	
59	+	+	-	-	-	
60	+	+	-	-	-	
61	+	+	-	-	-	Sample taken at 6½ miles. Exposure to sunlight. <i>No Pollution.</i>
62	+	-	+	+	-	
63	+	+	-	-	-	
64	+	+	-	-	-	
65	+	+	-	-	-	
66	+	+	-	-	+	
67	+	-	+	+	-	
68	+	+	-	-	-	



TABLE III.—*continued.*

	Lactose	Indol	Koser's citrate	Bacto- peptone citrate	Bacto- peptone tartrate	
70	+	+	—	—	—	Sample taken at 6½ miles. Exposure to sunlight. <i>No Pollution.</i>
71	+	—	+	+	+	
72	+	—	+	+	+	
73	+	—	+	+	+	
74	+	—	+	—	—	
75	+	+	—	—	—	
76	+	+	+	+	—	
77	+	+	+	+	+	
78	+	—	+	+	—	
79	+	—	+	+	—	
80	+	—	+	+	—	
81	+	+	—	—	—	Sample taken at 5½ miles. Below village. <i>Gross Pollution.</i>
82	+	+	—	—	—	
83	+	+	—	—	—	
84	+	+	—	—	—	
85	+	+	—	—	—	
86	+	+	—	—	—	
87	+	+	—	—	—	
88	+	+	—	—	—	
89	+	+	—	—	—	
90	+	+	—	—	—	
91	+	+	+	+	—	
92	+	+	—	—	—	
93	+	+	—	—	—	
94	+	+	—	—	—	
95	+	+	—	—	—	
96	+	+	—	—	—	
97	+	+	—	—	—	
98	+	+	—	—	—	
99	+	+	—	—	—	
100	+	+	—	—	—	
101	+	+	—	—	—	Sample taken at 1½ miles. <i>Exposed to sunlight.</i>
102	+	+	—	—	—	
103	+	+	—	—	—	
104	+	+	—	—	—	
105	+	+	+	+	—	
106	+	—	—	—	—	
107	+	+	—	—	—	
108	+	+	—	—	—	
109	+	+	—	—	—	
110	+	+	—	—	—	
111	+	+	—	—	—	
112	+	+	—	—	—	
113	+	—	+	+	+	
114	+	+	—	—	—	
115	+	+	—	—	—	
116	+	+	—	—	—	
117	+	+	—	—	—	
118	+	+	—	—	—	
119	+	+	—	—	—	
120	+	+	+	—	—	
121	+	+	—	—	—	Sample taken at 'terminus' of River. <i>Occasional Pollution above.</i>
122	+	+	—	—	—	
123	+	+	—	—	—	
124	+	+	—	—	—	
125	+	+	—	—	—	
126	+	+	—	—	—	
127	+	+	—	—	—	
128	+	+	—	—	—	
129	+	+	—	—	—	
130	+	+	+	+	—	
131	+	+	—	—	—	
132	+	+	—	—	—	
133	+	+	—	—	—	
134	+	+	—	—	—	
135	+	+	—	—	—	
136	+	+	—	+	—	
137	+	+	—	—	—	
138	+	+	—	+	—	
139	+	+	+	+	—	
140	+	+	—	—	—	

## LOPINOT RIVER, TRINIDAD.



TABLE illustrating the action of coli organisms isolated from various levels upon sodium citrate and tartrate.

Little or no evidence of pollution		Some pollution		Much pollution		Exposure to sunlight. No pollution		Gross pollution		Exposure to sunlight		Exposure to sunlight. Occasional pollution		Test substance
8½ miles		7½ miles		7½ miles		6½ miles		5½ miles		1½ miles		0 mile		
Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	
20	0	20	0	20	0	20	0	20	0	20	0	20	0	Lactose
6	14	20	0	19	1	10	10	20	0	18	2	20	0	Indol
19	1	7	13	1	19	11	9	1	19	3	17	2	18	Koser's citrate
8	12	2	18	0	20	5	15	0	20	1	19	0	20	Bacto-peptone tartrate
17	3	2	18	1	19	9	11	2	18	2	18	4	16	Bacto-peptone citrate

Pos. Koser's citrate

Pos. Bacto-peptone citrate

Pos. Bacto-peptone tartrate

Neg. Koser's citrate

Neg. Bacto-peptone citrate

Neg. Bacto-peptone tartrate

} = Utilisation of citrate and tartrate = non-faecal coli organism.

} = No utilisation of citrate and tartrate = faecal coli organism.

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## EXPLANATION OF PLATE XXV

- Fig. 1. No decomposition of tartrate. Measured volumes.  
Voluminous white flocculent precipitate; note  
'bursting' of precipitate by gas.
- Fig. 2. Decomposition of tartrate. Measured volumes. Small  
heavy granular precipitate.
- Fig. 3. No decomposition of citrate. Measured volumes.  
Voluminous white flocculent precipitate.

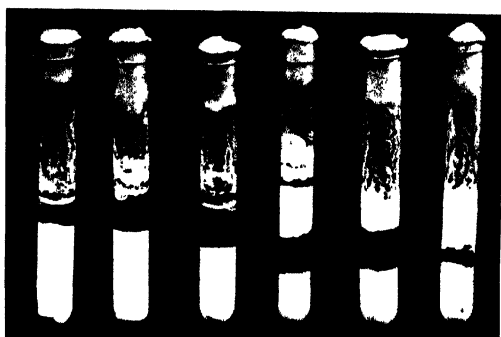


FIG. 1

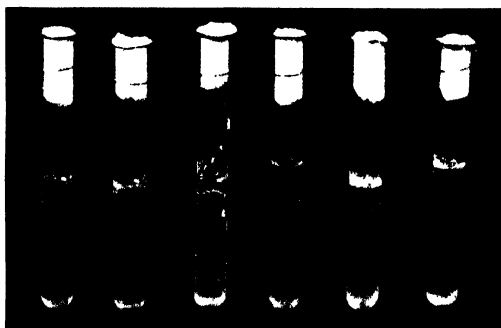


FIG. 2

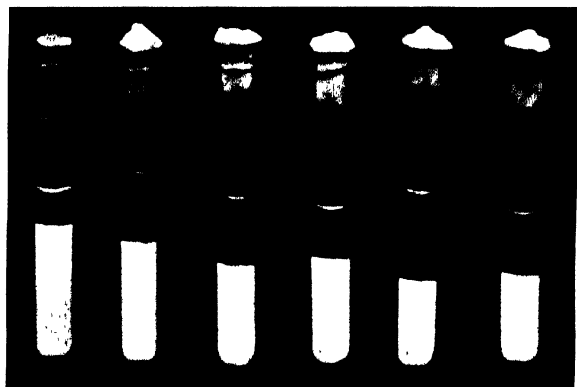


FIG. 3



**PLATE XVI**

## EXPLANATION OF PLATE XXVI

- Fig. 4. Decomposition of citrate. Measured volumes. Small heavy granular precipitate.
- Fig. 5. No decomposition of tartrate. Voluminous white flocculent precipitate formed.
- Fig. 6. Decomposition of tartrate. Small heavy granular precipitate formed.

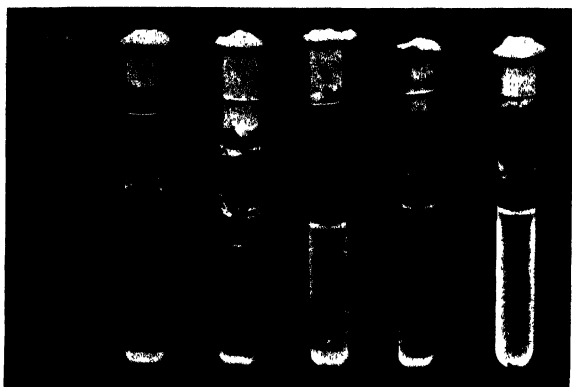


FIG. 4

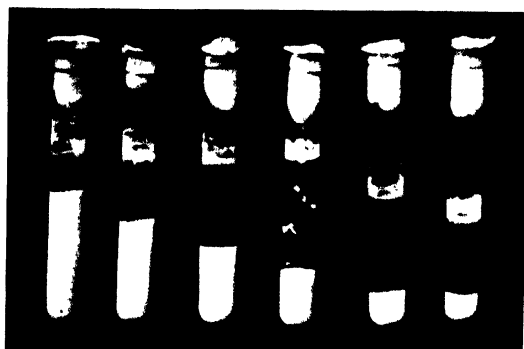


FIG. 5

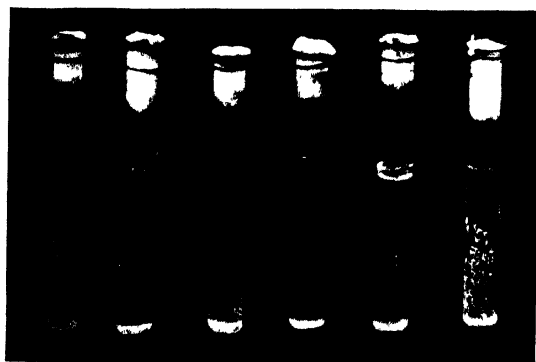


FIG. 6







## EXPLANATION OF PLATE XXVII

- Fig. 7. No decomposition of citrate. Voluminous white flocculent precipitate formed.
- Fig. 8. Decomposition of citrate. Small heavy granular precipitate formed.
- Fig. 9. To show solution of small granular precipitate seventeen days later decomposition of tartrate.



FIG. 7

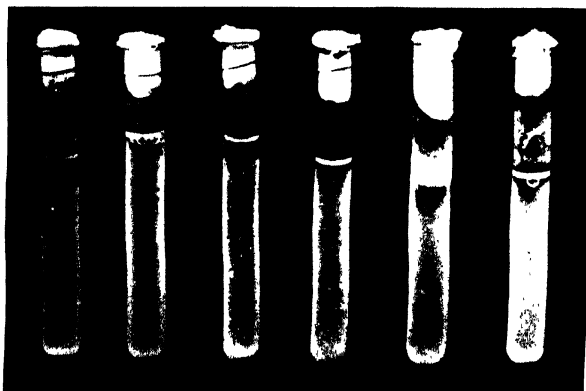


FIG. 8

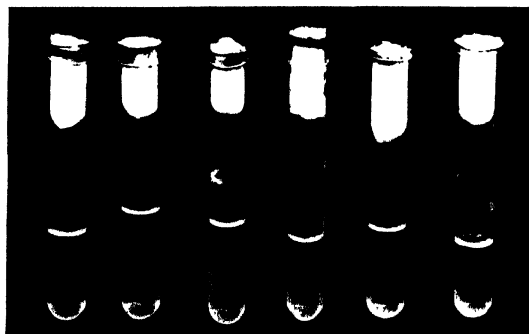


FIG. 9



# OBSERVATIONS ON *ENTAMOEBA* *HISTOLYTICA*

## II. LONGEVITY OF THE CYSTS *IN VITRO*, AND THEIR RESISTANCE TO HEAT AND TO VARIOUS DRUGS AND CHEMICALS

BY

WARRINGTON YORKE

AND

A. R. D. ADAMS

(Received for publication, 26 July, 1926)

Hitherto we have had no absolutely satisfactory criterion which would permit us to decide whether *E. histolytica* cysts are alive or dead. The usually accepted method of determining the vitality of cysts is their behaviour in dilute eosin solution. This test, which was apparently first employed for the purpose of deciding whether *E. histolytica* cysts were viable by Kuenen and Swellengrebel (1913), is based on the hypothesis that dead cysts stain with weak solutions of eosin in water (1 in 1,000) and that living cysts do not. Although it was impossible to determine whether this hypothesis was wholly correct, it was generally accepted that those cysts which stained were certainly dead, but whether all those which did not stain were alive seemed to be a much more doubtful matter.

Another possible method for determining the vitality of *E. histolytica* cysts is to feed them to cats, but apart from the enormous sacrifice of animals which such a method would entail in an extensive investigation on the vitality of the cysts, and the time which it would occupy, the procedure is, as Kuenen and Swellengrebel have pointed out, so often attended by negative results, even when quite fresh material is used, as to be hardly suitable for this purpose. Quite recently Sellards and Theiler (1924) have shown that cats can be infected by intrarectal injection of *E. histolytica* cysts, but the use of this technique for determining the vitality of cysts is open to the same objections.

In a previous paper (1926) we have described a method of obtaining cultures of *E. histolytica* from cysts. This method, which has in our hands proved invariably successful, seems admirably adapted for testing the vitality of *E. histolytica* cysts, and in the present work we have employed it for determining their vitality in faeces kept *in vitro* for varying lengths of time, and under various conditions, and for examining the effect on the cysts of heat, and of various drugs and chemicals.

#### LONGEVITY OF *E. HISTOLYTICA* CYSTS *IN VITRO*.

This is, from the public health point of view, a subject of fundamental importance, but owing to the hitherto inadequate and inaccurate methods available for deciding whether *E. histolytica* cysts are viable, it is not surprising that the data obtained are somewhat scanty and highly conflicting.

Kuenen and Swellengrebel kept infected faeces at laboratory (27 to 30° C.) and at incubator (37° C.) temperatures, and found that after three days all the cysts had disappeared from the specimen kept at 37° C. ; in that kept at 27 to 30° C. all the cysts were alive after three days, but about half of them were dead by the fourth day, and all by the ninth day. The cysts from another portion of the same faeces were separated, so far as possible, from faecal material, by washing and centrifuging, and allowed to stand at 27 to 30° C. in water ; under these conditions practically all the cysts were found to be alive on the ninth day : subsequently enormous bacterial growth occurred, and this is stated quickly to have destroyed the cysts, so that hardly any living individuals were to be found by the thirteenth day ; nevertheless, a few are stated to have been still alive after 29 days. These conclusions were based on the eosin criterion.

Penfold, Woodcock, and Drew (1916), who judged of the viability of the cysts by their power to excyst in the presence of *liquor pancreaticus*, state that they were able to 'keep cysts in a very slowly running current of water for 15 days and to ascertain that certainly some were alive at the end of this period,' and they infer 'that water which has been contaminated with cyst-containing faeces may remain a source of infection for a considerable period.' Wenyon and O'Connor (1917) employing the eosin test for viability

write, that 'cysts will survive for over 30 days in water,' and that 'apparently the cysts survive best if there is considerable dilution of faeces with water, so that intense bacterial or fungoid overgrowth does not take place.'

Dobell (1919) writes as follows :—'The cysts of *E. histolytica* will survive for several weeks outside the body of man, if they are kept moist and cool. They will live in damp faeces or in water without showing any conspicuous change save the loss of their chromatoid bodies. As a rule, if the cysts are kept under observation, it will be found that some of them remain alive much longer than the others. In water or faeces some will usually be found dead at the end of a week, many more after the lapse of a fortnight, and after this period only isolated survivors will be discoverable. The longest time of survival which I have observed is five weeks (cysts kept in water), but as a rule they will not live so long. Desiccation kills them immediately, and they degenerate much more rapidly at a high than at a low temperature. At body temperature they generally die within a few days at most. Degeneration of the cysts is readily recognizable. The nuclei first become unnaturally distinct in the fresh cysts—owing to the coagulation which occurs on the death of the protoplasm—and then break up. As the cysts die they also become permeable to aqueous solutions of various stains (eosin, etc.). The cytoplasm becomes vacuolated, and finally disintegrates.'

Boeck (1921b), accepting the eosin-solution supplemented by morphological observations as the criterion of viability, states that when 'immersed in distilled water, contained in bottles and kept at a temperature of 12° to 22° C., cysts of *E. histolytica* were found viable at the end of 153 days'; and 'in eosin-stained wet preparations, sealed with vaseline, cysts of *E. histolytica* were viable at the end of 211 days.'

Summarising these records we find some indication that cysts live longer in water than in faeces, and that low temperatures are more favourable for longevity than are higher, or body temperatures. The actual period for which, under favourable conditions, the cysts will remain viable is stated to be very different by the various investigators, e.g., Kuenen and Swellengrebel found a few alive after 29 days, Wenyon and O'Connor state they will survive for over 30 days, Dobell found some alive up to five weeks, and Boeck states



that they may live for such lengthy periods as 153 and 211 days.

In re-investigating the subject we decided to ascertain the longevity of *E. histolytica* cysts, not only in the original faecal mass kept at 0° C. and at laboratory temperature (16 to 20° C.), but also in water suspensions—prepared by both of the methods described in our previous paper (1926)—kept at similar temperatures. In order to prevent bacterial overgrowth the suspensions were washed every three or four days by centrifuging, removing the supernatant fluid, and replacing it by fresh water or saline. The suspensions were tested for the presence of live cysts by sowing periodically five or six drops of the centrifuged deposit on L.E.S. medium, and examining the culture for vegetative *Entamoebae* in the manner previously described, after incubation at 37° C. for 16 to 20 hours. The cysts in the faecal mass were examined, not by sowing some of the faecal mass itself on L.E.S. medium, but by preparing from a small portion of it a washed concentrated suspension of cysts and sowing a few drops of this as described above: this procedure was, for obvious reasons, found to be a more sensitive test of the presence of live cysts than the direct sowing of the faecal material.

Experiments of this nature were performed on a number of occasions, and with infected faeces from several different individuals; in all cases the results were very similar.

It will be observed from Table I, which sets forth the results obtained in one experiment of this nature, that in each case the ice-chest specimen survives the longer; and again that the longevity of the cysts is greater in the washed suspension than in the original faecal mass. The maximum period for which we have found cysts to remain viable, at room temperatures, in the faecal mass has never exceeded nine days, and in washed suspensions, ten days; whereas a saline or water suspension maintained at 0° C. has been found to contain a few viable cysts up to the seventeenth day. It should be noted that in the later periods the cultures were not nearly as luxuriant as on the first few days, and eventually prolonged search was necessary to determine whether they were actually positive or negative.

TABLE I.

Showing the result of culturing *E. histolytica* cysts after storing *in vitro* under various conditions for varying periods of time.

Time in days	Presence or absence of vegetative <i>Entamoebae</i> in 16 hour-old cultures			
	Infected faeces stored at 0° C.	Infected faeces stored at 16 to 20° C.	Washed concentrated suspension of cysts stored at 0° C.	Washed concentrated suspension of cysts stored at 16 to 20° C.
1	+	+	+	+
4	+	+	+	+
7	+	+	+	+
9	+	-	+	+
10	-	-	+	+
11	-	-	+	-
15	-	-	+	-
17	-	-	+	-
21	-	-	-	-

### RESISTANCE OF *E. HISTOLYTICA* CYSTS TO HEAT

Apparently the only observations on this subject are those of Boeck (1921a), who, using dilute eosin as a test of viability, found that the thermal death point of *E. histolytica* cysts was 68° C.

Our investigations on the point were conducted as follows:—

A washed concentrated suspension of *E. histolytica* cysts was prepared by the sugar method (*vide* previous paper) and two drops of the centrifuged deposit containing very numerous cysts (50 or more to the microscope field, A obj. No. 4 oc.) were added to a series of centrifuge tubes each containing 5 c.c. of water. The tubes were then heated in water baths at various temperatures for 5 minutes and 30 minutes respectively. After the lapse of these periods the tubes were centrifuged, the deposit sown on L.E.S. medium, and the cultures examined for vegetative *Entamoebae* after incubation for 16 to 20 hours.

The results are given in Table II from which it will be seen that, for the periods in question, *E. histolytica* cysts withstand a temperature of 45° C., but are all killed by a temperature of 50° C.

TABLE II.

Showing the result of culturing *E. histolytica* cysts, which had been exposed to various temperatures for 5 minutes and 30 minutes respectively.

Temperature to which cysts were exposed	Presence or absence of vegetative <i>Entamoebae</i> in 16 hour-old cultures.	
	Length of time for which cysts were exposed to various temperatures	
	5 minutes	30 minutes
40° C.	++	++
45° C.	++	++
50° C.	—	—
55° C.	—	—
58° C.	—	—
60° C.	—	—

#### THE RESISTANCE OF *E. HISTOLYTICA* CYSTS TO VARIOUS DRUGS AND CHEMICALS

Kuenen and Swellengrebel, by means of the dilute eosin test for viability, found that a solution of sublimate (1 in 1,000) killed *E. histolytica* cysts in four hours, creolin (1 in 250) killed them in five to ten minutes, and 50 per cent. alcohol or Schaudinn's sublimate alcohol solution killed them immediately; on the contrary it is stated that the cysts were very resistant to emetin hydrochloride, and that they survived exposure to a 10 per cent. solution of formalin for a few minutes.

Wenyon and O'Connor, also relying on eosin as a test for viability, found that acid sodium sulphate and chlorinated lime tabloids (B. W. & Co.) as used for water sterilization, had no action on *E. histolytica* cysts; that they withstood 1 in 200 emetin hydrochloride for nine hours; but were killed immediately by cresol (1 in 20), in one minute by a dilution of 1 in 30, and in half-an-hour by 1 in 100; carbolic acid (1 in 40) killed them in fifteen minutes, and in a dilution of 1 in 100 in seven hours: cysts exposed to 1 per cent. formalin for four hours did not stain with eosin, but were much shrunken and distorted, and had every appearance of being dead.

Boeck and Stiles (1923), likewise basing their observations on the reliability of the eosin test as a criterion of viability, make the

astonishing statement that 'The cysts of *E. histolytica* appear to be viable as long as five days in 5 per cent. formalin.'

In order to re-investigate this subject we conducted experiments of the following nature :—

A washed concentrated suspension of *E. histolytica* cysts was prepared by the sugar method, and two drops of the centrifuged deposit added to 5 c.c. of the fluid to be tested, and allowed to remain there for 30 minutes at either laboratory temperature (20° to 25°C.) or at 37°C. The cysts were then precipitated by means of the centrifuge, and after removal of the supernatant fluid washed four times in water to get rid of all trace of the chemical, and then sown on L.E.S. medium, which subsequently was examined for active *Entamoebae* after 16 to 20 hours incubation.

Such a method of testing the effect of drugs and chemicals on *E. histolytica* cysts is, of course, far more drastic than merely adding the disinfectant to the infected faeces, as although the couple of drops of washed concentrated suspension contained large numbers of cysts, yet the other organic matter present was relatively very small in amount, and the total quantity used was practically insignificant as compared with the relatively enormous volume of the disinfectant. The action of a considerable number of drugs and chemicals, in various strengths, was tested on several occasions with the results shown in Table III. It will be seen that although *E. histolytica* cysts are remarkably resistant to such substances as emetin hydrochloride, 'Yatren,' and hydrochloric acid, they are nevertheless fairly sensitive to such common disinfectants as mercuric chloride, formaldehyde, carbolic acid, and lysol. The result of testing the effect of chlorine (and hypochlorous acid) is interesting and important in that although a dilution of 1 in 64 of a saturated aqueous solution of chlorine (representing about .01 per cent. of chlorine) is sufficient to destroy all the cysts, yet this concentration is infinitely greater than that used to sterilize water of bacteria. The ordinary procedure of adding chlorinated lime to water in quantities known to be sufficient to destroy all *Bacillus coli* is without effect on *E. histolytica* cysts, and in fact the addition of this substance to water contaminated with *E. histolytica* cysts is, for practical purposes, completely useless.

TABLE III.

Showing the result of culturing *E. bistolytica* cysts which had been exposed to various chemicals for 30 minutes at laboratory temperature and at 37° C. respectively.

Chemicals to which cysts were exposed	Concentration	Presence or absence of vegetative <i>Entamoeba</i> in 16 hour-old cultures	
		Temperature at which cysts were exposed to chemicals	
		Lab. temperature (20 to 25° C.)	37° C.
H Cl ... ..	0.2 per cent. ... ..	++	++
	1.0 " ... ..	++	++
	5.0 " ... ..	++	-
	7.5 " ... ..	-	-
	10.0 " ... ..	-	-
NaOH ... ..	0.2 per cent. ... ..	++	++
	1.0 " ... ..	+	+
	2.5 " ... ..	-	-
	5.0 " ... ..	-	-
Cl ... ..	*Sat. sol. in water ...	-	-
	1/2 " " ... ..	-	-
	1/4 " " ... ..	-	-
	1/8 " " ... ..	-	-
	1/16 " " ... ..	-	-
	1/64 " " ... ..	-	-
	1/320 " " ... ..	+	-
Chlorinated lime tablet (B. W. & Co.)	†1 tablet in 65 c.c. water ...	+	-
	1 " 325 c.c. " ...	++	+
	1 " 650 c.c. " ...	++	++
	1 " 6500 c.c. " ...	++	++
Miltoni ... ..	100.0 per cent. ... ..	-	-
	10.0 " ... ..	-	-
	5.0 " ... ..	-	-
	2.5 " ... ..	-	-
	1.0 " ... ..	+-	+-
	0.5 " ... ..	+-	+-
HgCl <sub>2</sub> ... ..	1 in 500 ... ..	-	...
	1 in 2,500 ... ..	-	...
	1 in 12,500 ... ..	+	...
	1 in 100,000 ... ..	++	...
Pot. permanganate	1.0 per cent. ... ..	+	+
	0.2 " ... ..	++	++
	0.02 " ... ..	++	++
Formaldehyde ...	2.5 per cent. ... ..	-	-
	0.5 " ... ..	-	-
	0.2 " ... ..	++	+
	0.1 " ... ..	++	++
	0.05 " ... ..	++	++
Carbolic acid ...	2.5 per cent. ... ..	-	-
	1.0 " ... ..	-	-
	0.5 " ... ..	++	++
	0.1 " ... ..	++	++

TABLE III—*contd.*

Showing the result of culturing *E. histolytica* cysts which had been exposed to various chemicals for 30 minutes at laboratory temperature and at 37° C. respectively.

Chemicals to which cysts were exposed	Concentration	Presence or absence of vegetative <i>Entamoebae</i> in 16 hour-old cultures	
		Temperature at which cysts were exposed to chemicals	
		Lab. temperature (20 to 25° C.)	37° C.
Lysol ... ..	2'5 per cent. ... ..	—	—
	1'0 " ... ..	—	—
	0'5 " ... ..	+	+-
	0'1 " ... ..	++	++
Yatren ... ..	5'0 per cent. ... ..	+	+
	2'5 " ... ..	++	++
	1'0 " ... ..	++	++
	0'1 " ... ..	++	++
Emetin H Cl ... ..	5'0 per cent. ... ..	++	++
	2'5 " ... ..	++	++
	1'0 " ... ..	++	++
	0'5 " ... ..	++	++

++ Signifies Numerous *Entamoebae*.

+ " Scanty *Entamoebae*.

+- " *Entamoebae* present on some occasions and absent on others.

— " Consistently negative.

\* A saturated solution of chlorine in water contains at 24° C. approximately 0'7 per cent. of chlorine by weight.

† A tabloid (B. W. & Co.) of chlorinated lime is equivalent to 0'065 gm. of chlorine.

‡ Stated to contain sodium hypochlorite with a relatively high percentage of hypochlorous acid; the available chlorine is 1'05 per cent.

## SUMMARY

1. The work described in this paper indicates that the eosin reaction is not an entirely reliable criterion of viability in so far as *E. histolytica* cysts are concerned; whilst those cysts which stain with dilute eosin are almost certainly dead, it is by no means true that all those which do not stain are alive.

2. A more reliable and satisfactory method of determining the viability of *E. histolytica* cysts, based on the fact that they can be readily cultured *in vitro*, is described; and the following information regarding their longevity, thermal death point, and resistance to chemicals and drugs, has been obtained.

3. *E. histolytica* cysts commence to die fairly rapidly in faeces which has been kept at laboratory temperature (16 to 20° C.) for 3 or 4 days, and all are dead within about 10 days; approximately the same result is obtained when the faeces is kept at 0° C. in the ice-chest.

4. Washed suspensions of *E. histolytica* cysts in water live rather longer, more especially when kept at 0° C.: but even under these conditions live cysts are not found after three weeks.

5. *E. histolytica* cysts survive a temperature of 45° C. for 30 minutes, but are killed within five minutes by a temperature of 50° C.

6. *E. histolytica* cysts are remarkably resistant to emetin and to 'Yatren'; and relatively so to hydrochloride acid and chlorine, the last-named, in strengths far in excess of that used in the bacteriological sterilization of water, having no effect on the cysts. The lethal strengths of solutions of such substances as mercuric chloride, potassium permanganate, formaldehyde, lysol, carbolic acid, and the proprietary preparation 'Milton' have been ascertained.

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# THE MOSQUITO INFECTIVITY OF *P. VIVAX* AFTER PROLONGED SOJOURN IN THE HUMAN HOST

BY  
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AND  
W. REES WRIGHT

(Received for publication, 29 July, 1926)

In the now extensive literature relating to the malaria treatment of general paralysis, the statement is not infrequently encountered that maintenance in the human host for prolonged periods, by direct inoculation of infective blood from one individual to another, modifies the malaria parasite in certain important respects.

It has been pointed out by Duke (1923) that *Trypanosoma brucei* ceases to infect *Glossina* after it has been passed through a series of about twenty vertebrate hosts; and it is now likewise claimed (Gerstmann, Barzilai-Vivaldi, Plehn, and others) that strains of *Plasmodium vivax* which have been maintained in man for prolonged periods have lost their capacity to infect anopheles.

In previous papers (Yorke and Macfie, 1924, and Yorke, 1925) reference is made to the fact that a strain of *Plasmodium vivax* maintained by direct passage in the human host since September, 1922—partly at Whittingham, and partly at Sheffield, mental hospitals—was still capable of infecting *A. maculipennis* at various passages up to the forty-first.

In March, 1926, after the strain had been maintained in the human host for three and a half years, its capacity to infect *A. maculipennis* was again examined. Forty-seven mosquitos were allowed to feed three times on a patient of the fifty-third passage, and once on a patient of the fifty-fourth passage. Fifteen days after the first feed, the mosquitos were divided into four groups, each of which was fed on a general paralytic; all four patients became infected



with malaria. Twenty-three of the mosquitos, which lived for longer than a week after the first feed, were dissected, and of these nineteen were found to be infected—three with oocysts only, and sixteen with sporozoites in the salivary glands.

This observation shows that the strain in question had preserved unimpaired its power to infect mosquitos after fifty-three or fifty-four direct passages through man during a period of three and a half years.

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1926	Hawe, A. J.

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# ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

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Articles for publication should not exceed twenty-five pages of the *Annals*, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to: The Editors, School of Tropical Medicine, The University, Liverpool.

Illustrations for text figures or charts should be drawn clearly and firmly in Indian ink, if possible on Bristol board. N.B.—*Blue or other coloured ruling in square lines cannot be reproduced*

All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

Plates and <sup>weak</sup> illustrations should be accompanied by short explanations

References to authors in the text must be made in the following way:—‘According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.’ The references should be collected in alphabetical order of authors’ surnames at the end of the paper, and arranged in the following way:—

ROBINSON, S. (1914). ‘The spleen in malaria.’ *Annals of Nosology*, Vol. XX, pp 20-25

SMITH, J. (1900). ‘Enlargement of the spleen in malaria.’ *Journal of Pathometry*, Vol. I, pp. 1-20.

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# HISTORY OF AN OUTBREAK OF RHODESIAN SLEEPING SICKNESS IN THE UFIPA DISTRICT OF TANGANYIKA TERRITORY WITH SHORT NOTES ON CASES AND TREATMENT

BY

GEORGE MACLEAN

MEDICAL OFFICER, TANGANYIKA TERRITORY

*(Received for publication 5 July, 1926)*

## I. INTRODUCTION

The outbreak to be described occurred in a portion of a large open forest, which is said to extend, with few breaks, over an area of 50,000 square miles. In the part surveyed the ground is for the most part gently undulating, though hilly in places; the average altitude is about 3,000 feet above sea level. The type of forest is that generally known as 'Miombo,' though true Miombo is only one of the many species of trees present. The forest affords light shade and it is rare to meet with much undergrowth.

Only two rivers of size traverse the area, namely, the Rungwa, with its tributaries, the Lugombe, Wogo, etc., and the Ugalla or Shama. These rivers, which are several hundred yards wide in the height of the rains, are reduced to series of stagnant pools towards the end of the dry season. During this season these pools form practically the only natural water supply of the area. Certain of these pools abound in fish which forms an important source of native food supply.

The people live in small scattered clearings where the soil is fertile and water supply easily obtained. These clearings are frequently only a few acres in extent. A fifty-acre clearing is considered a large one, and half a square mile is exceptional.

'A village' may consist of anything from one to forty, rarely more, houses; an average village consists of five to ten.



Only one species of tse-tse was met with, viz., *G. morsitans*. This fly, which is scanty except at certain centres, from about the middle of the dry season (August-September) to the beginning of the rains (November-December), is practically co-extensive with the forest towards the end of the rains and the beginning of the dry season (May-June).

Other biting flies met with were several species of *Tabanus*, *Haematopota*, and, in some places, *Chrysops*.

Game is fairly abundant, the commonest being—Eland, Roan, Bush-Buck, Oribi and other small buck, Baboons, Zebra, Giraffe, Bush-pig and Wart-hog; and in some places, Sable, Lesser Kudu, Water-buck, Buffalo, and Elephant. Leopards and Hyaenas are also common.

Those most frequently met with near human habitations are: Roan, Bush-buck, Oribi and allied species, Bush-pig, Wart-hog, Baboons.

## II. THE HISTORY OF THE OUTBREAK

Whether the disease has been endemic for an indefinite period or whether it is of recent introduction is uncertain. The natives, almost unanimously, regard it as a new disease, stating that it had its starting-point at Tumbu (E<sub>3</sub>, A6), whence it radiated to other parts of the district. On the other hand, in the absence of a survey,—and no record of such a survey has been found—sporadic cases could easily occur, unrecognised. It is a common experience to find natives confusing Sleeping Sickness with other diseases, particularly with *Ankylostomiasis*—a disease which is endemic in the district.

The question of endemicity, then, still remains open; on the other hand, the present high incidence appears to be something entirely new. Apart from the natives' insistence on its being a new disease, it will be seen later that the level—or anything approaching it—of this incidence could not be maintained for many years without depopulating a whole country-side.

The recent history is fairly clear. It is known that deaths occurred from a disease, characterised by swelling of the legs, as early as 1915, in the vicinity of the Mtäte River (E<sub>3</sub>, A6 and 11), but the information is vague and it may or may not have been an early

manifestation of the present outbreak. Nothing more is definitely known until in 1920, or 1921, a fatal disease established itself at Tumbu. This disease was characterised by emaciation, swelling of the legs, and fatal termination. There were said to have been sixteen families in Tumbu before the infection came ; within a year all these either died or escaped to neighbouring villages.

The next village to be attacked was Kilundu (E<sub>3</sub>, A11) and it suffered a similar fate. Some time later cases occurred at the group of settlements at Simbo (E<sub>2</sub>, D12). Here the havoc was not so complete and a large proportion of the population still remains. There appear to have been cases in Simbo early in 1924, and two cases from its vicinity were found positive in January, 1925.

On the northern side of the Rungwa River the first village known to be involved was Ilundu (E<sub>2</sub>, D8d), which, apparently, had a good deal of intercommunication with Tumbu. In the course of a year or so, at least ten people (probably not all Sleeping Sickness) out of a population of forty or fifty died. The remainder took refuge elsewhere, at Ilola (E<sub>2</sub>, D8 and E<sub>3</sub>, A5), Katutu (E<sub>3</sub>, A5), Isote (E<sub>3</sub>, A1), and, possibly, other places.

Among those who died at Ilundu was a woman who used to live at Tumbu : she left there for Ilundu, with symptoms of Sleeping Sickness, when Tumbu was evacuated. One at least of the refugees from Ilundu is stated to have died at Ilola, with symptoms of Sleeping Sickness. Whether this was the first case at Ilola, or when the first case occurred is uncertain, but, apparently, not later than 1923.

About the same time cases occurred at Mbao's (E<sub>2</sub>, D8). In that year or early in 1924 two deaths from the same disease occurred at Ilinga (E<sub>3</sub>, A2). One of these was a native of Ilinga, who developed symptoms after a visit to relatives in Ilola, while the other was a refugee from the latter place.

At what date the disease reached Mwanda (E<sub>2</sub>, D11 and 12) is uncertain, but it was well established both there and at Kianga's (E<sub>2</sub>, D8) by April, 1924. Later it reached Itetemya (E<sub>2</sub>, D7), Hatagelo (E<sub>2</sub>, D8), Kasamia's (E<sub>2</sub>, D7), and Chandarua's (E<sub>2</sub>, D11). Apparently there was constant intercommunication between these different settlements, and ample opportunities for infection by direct or indirect transmission.

During these months of 1924 the disease reached epidemic

proportions. At Ilola, of about thirty-five or thirty-six families living in 1923, only eight were left in November, 1924; at Mwanda only two out of sixty-four or sixty-five; while Kianga's, with one family, and Mbao's, with eight families, were completely depopulated.

What proportion died is uncertain, but it is stated that the majority did so and that only a small minority escaped.

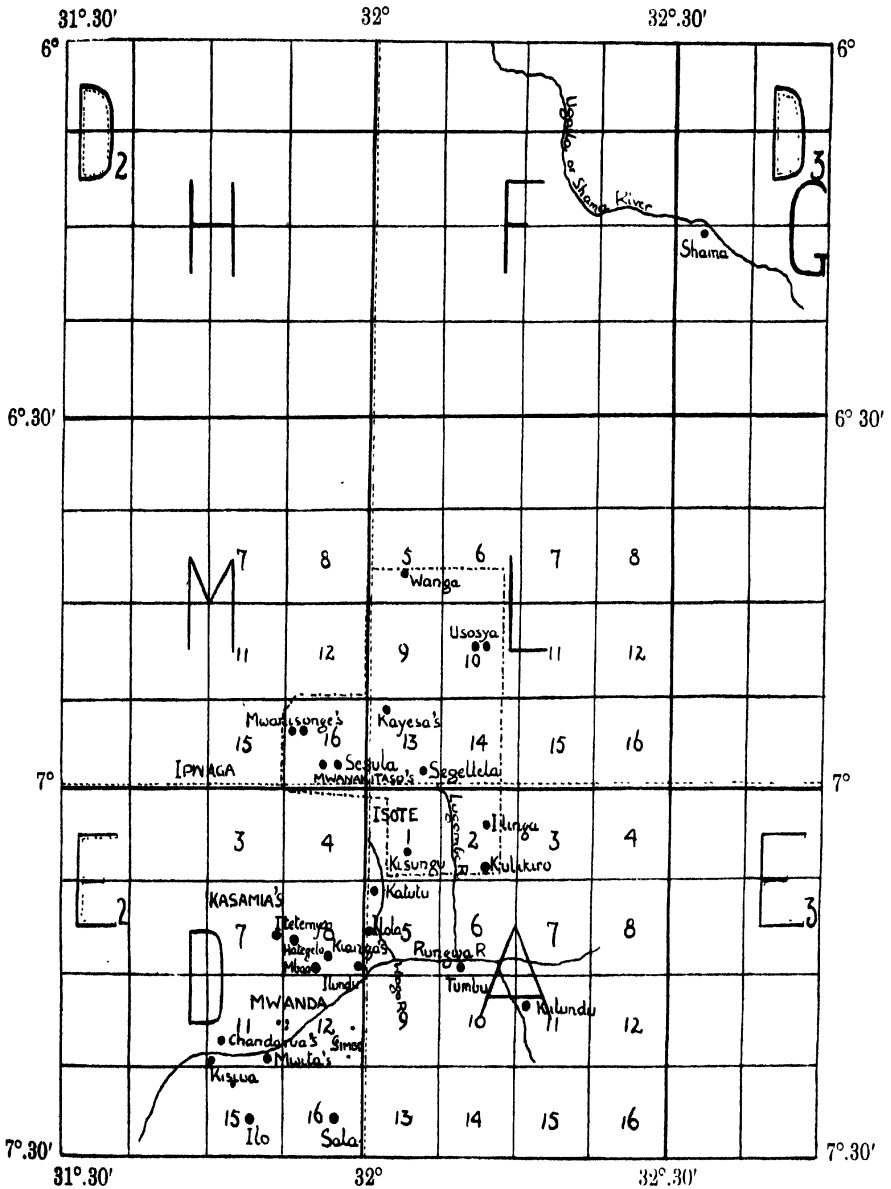
A number of the now panic-stricken refugees went Northward to Isote (E<sub>8</sub>, A1), Kisungu (E<sub>8</sub>, A1), Mwanakitaso's (E<sub>8</sub>, A1, etc.) and elsewhere. Others from Chandarua's went South of the Rungwa River to Mwita's (E<sub>8</sub>, D11) and Bigiri's (E<sub>8</sub>, D) and some of the refugees became ill at both these places. Subsequently some of the inhabitants of Mwita's became sick and two of these sick removed to Kisiwa (E<sub>8</sub>, D 11) where they were found suffering from Sleeping Sickness, in 1925.

Of the refugees who went North some became ill at Kisungu and other places. Natives of Kisungu now became infected and were found suffering from Sleeping Sickness late in 1924. At Ilinga, a few miles away, cases had occurred as already stated. Quite apart from the fact that there must have been many refugees whose movements are unknown, there was enough intercommunication between the places above mentioned and their neighbours to account for the cases that subsequently occurred in the surrounding villages (see map).

The next important stampede occurred from Ilinga in the latter part of 1924. After the deaths of four or five people in the course of a few months (some of them apparently not Sleeping Sickness), a large number of the people fled, some to neighbouring settlements like Mwanakitaso's and Kulikiro (E<sub>8</sub>, A2), others further afield to Ilo (E<sub>8</sub>, D15), Shama (D<sub>8</sub>, G9), Kiwere (D<sub>8</sub>, N10), etc. Some of these refugees were subsequently found suffering from Sleeping Sickness at Mwanakitaso's, Kulikiro, Shama, and Ilo. Some time before this stampede some persons from Sala (E<sub>8</sub>, D16) visited Ilinga at different times (possibly passing through infected villages on the way), and on returning home developed Sleeping Sickness.

Survey was commenced in November, 1924. The history prior to that date is based almost entirely on native information collected between November, 1924, and January, 1926. Cases of Sleeping

## UFIPA SLEEPING SICKNESS AREA.



Sickness mentioned above, which occurred after the commencement of the survey were, in almost every instance, diagnosed microscopically.

Outbreaks occurred at the same time in other parts of the same forest, but as a connection between them and this has not, so far, been definitely established, they are not included in this paper.

The history has been given in some detail because it appears to show the importance of the human agent in spreading the disease when the incidence is high. Cases have occurred which could not be traced to a human infected focus, but it is not improbable that a more thorough knowledge of their movements would elicit a connection with a human source. The history also illustrates the devastation that can be made in a sparse population by this form of Sleeping Sickness before it undergoes spontaneous arrest.

### III. CAUSES OF THE OUTBREAK

These are unknown but certain disturbing factors may be mentioned. The most important of these appear to be the late War and Influenza. First the former and then the latter led to considerable disorganisation and movements of the population. No aetiological influence, however, has been conclusively traced to either of them.

Shortage of food is of almost annual occurrence in some part or other of the forest and what influence it may have had is more likely to have shown itself in helping to propagate an established disease than in paving the way for a new outbreak.

### IV. THE INCIDENCE OF THE DISEASE

The following figures are based on observations made in the Baula Sultanate (excluding Ipwaga) with a few neighbouring villages which, geographically, form part of it, the whole occupying an area of 500 square miles. This was the part most thoroughly surveyed, and, possibly, also that most heavily infected.

Settlement	Estimated population, November, 1924	No. of positive cases between November, 1924, and 31st December, 1925
Ilinga ... ..	34	12
Kayesa's ... ..	50	2
Mwanetsonge's ... ..	33	3
Mwanakitaso's ... ..	60	21
Segellela ... ..	61	1 (a refugee)
Sesula ... ..	80	7
Usosya ... ..	90	5
Wanga ... ..	33	0
Kisungu ... ..	28	8
Kulikiro ... ..	23	3
Mtalaza ... ..	21	0
Other village ... ..	50	1
TOTAL ... ..	563	62 (excluding 1 refugee)

Highest incidence in one settlement, 35 per cent.

Incidence for Sultanate, 11 per cent.

(These 'Settlements' are groups of villages in many instances separated by forest)

## V. DIAGNOSIS

Rhodesian Sleeping Sickness is based—

(a) On the comparative acuteness of the disease, the duration of untreated cases being usually not longer than about six months.

(b) The finding of 'posterior nuclear forms' in an infected rat.

## VI. THE EARLY STAGES OF THE DISEASE

The following case may be of interest as illustrating the earliest stages from the probable time of infection.

The patient, a personal boy, lived at the coast (Dar-es-Salaam) before entering the tse-tse forest, and so far as is known had not been near an endemic locality for years.

He entered the tse-tse forest on 1st November, 1924, and reached the vicinity of an infected village for the first time on 11.11.24. He remained in good health till 21.12.24, in the meantime doing his ordinary work and frequently doing long marches.

On 21.12.24 he became acutely ill with fever and general malaise.

On 22.12.24 his condition was unchanged; trypanosomes were found in his blood.

On 23.12.24 he had pain in the side of the chest and cough, but no physical signs in chest.

On 25.12.24 he was improved but had anorexia.

4.1.25. Gradually improving and appetite returning. Walking about a little. Trypanosomes again found in the blood.

Glands not enlarged except axillary which were distinctly palpable.

Treatment with 'Bayer 205' commenced.

'Bayer' in 1.2 grm. doses was repeated on 10th and 29th days, all intramuscularly. He had vomiting and diarrhoea and, subsequently, an abscess after his first injection, but after his second he made a rapid and uninterrupted recovery.

He took up his ordinary duties again on 1.4.25.

Subsequently he remained in excellent health, doing strenuous work almost continuously, until January, 1926, when he unfortunately sustained a fatal injury.

## VII. TREATMENT

### (1) 'BAYER 205.'

Of twenty-seven cases treated between November, 1924, and March, 1925, fourteen still (March, 1926) survive, eight of them having remained in good health, without further treatment, since December, 1924. One of these eight was ill for two months, the other seven—with the possible exception of one, who did not give a clear history—were early cases, symptoms being of one month's duration or less before treatment was commenced.

All relapsed cases still surviving—with the possible exception of one, who did not give a clear history—were more advanced cases who had symptoms for two or more months before commencing treatment. Two of these relapsed cases are now in good health after Tryparsamide treatment.

One more case was alive, but with parasites in his blood, in December, 1925. He has not been treated since.

Of twelve cases that died, six only had a full course of treatment—a full course being three to four, or more, injections in 1·1-1·2 gramme doses for adults, and proportionately small doses for children—the other six who died did so either before the course could be completed, or after having, for various reasons, discontinued treatment. Of the six fully-treated, three had symptoms for two or more months before commencing 'Bayer'; the other three—two of whom were children under ten years—had symptoms for about a month. (Two of the three advanced cases had a further course of 'Bayer,' which resulted in temporary improvement.)

One case not included in the above series, who commenced treatment a fortnight after onset of symptoms, remained in excellent health for a year, when he sustained a fatal injury (see para 6).

*Period Intervening Before Relapse.* Symptoms, with negative blood, were met with as early as four months after completion of treatment. In other cases parasites were found in the blood, without symptoms, nine months after completion of treatment. (As these cases were not under continuous observation, parasites may have been in the blood much earlier than was observed.)

When 'Bayer' is used for sterilisation of the blood in advanced cases it must be remembered that trypanosomes may re-appear long before there is any definite clinical evidence of relapse, and as the parasites are still, presumably, infective, these cases may become a source of danger to others unless re-sterilisation of the blood is effected.

*Toxic Effects of 'Bayer.'* Albuminurea is a frequent sequel, but, in its immediate effects at least, not usually serious.

The following case suggests that the drug may, sometimes, affect the skin.

The patient, a young man, commenced treatment with 'Bayer' about a month after onset of his symptoms.

1.12.24. 1·2 grm. 'Bayer' was given intramuscularly.

14.12.24. 1·2 grm. 'Bayer' was given intramuscularly.

31.12.24. Reported with a vesicular rash over face, trunk, and limbs, and a diffuse stomatitis. Rash was said to have been present a few days only. Specimen of urine was not obtained.

15.1.25. Improved. Trace of albumen in urine.



25.1.25. Large amount of albumen in urine. Rash and stomatitis almost subsided.

Albuminurea persisted till February, 1925, but there was no recurrence of rash. Case was then lost sight of for some months.

Another series of cases treated during the latter part of 1925 is under observation.

## (2) TRYPARSAMIDE.

Of two cases, both women, who commenced treatment, 3 grammes every week, for eight weeks, in November, 1924, one is still well. Her blood remained negative till March, 1925, when she was temporarily lost sight of. Seen again in December, 1925, parasites were found in the blood, though the patient was in fairly good health and working regularly. Another course of treatment was then given.

The other case whose blood was negative after the first injection showed parasites again after the eighth. She died of Pneumonia shortly after completing her course of treatment. (In these two cases boiled solutions of the drug were used in the last two injections.)

A third case, treated in December, 1924, died suddenly a few days after her second injection.

A fourth case, in whose blood parasites were found only after frequent and prolonged searches, had his blood swarming with trypanosomes seven days after an injection of 4 grammes of Tryparsamide. He subsequently improved but did not complete a regular course of treatment.

Of a series, subsequently tried with boiled solutions of the drug, the majority died before completing a course. A few did well, temporarily, remaining in good health for several months. These last were cases in which the disease was mild.

(3) It is not yet possible to make a fair comparison between 'Bayer 205' and Tryparsamide.

The latter, besides being unstable to high temperatures, and apparently, to certain filtered waters, requires more investigation on several points. For instance, the total dosage, which ordinarily is 24-32 grammes, may have to be considerably increased or, it may be, the best results may be obtained by combining it with another drug.

'Bayer 205' and Tryparsamide combined are now being tried in a series of cases.

### VIII. ACKNOWLEDGMENTS

I am indebted to Mr. G. W. Hatchell, Administrative Officer i/c Ufipa District, for collecting a mass of information bearing on the history of the outbreak. But for his assistance large gaps in the history would still remain unfilled ; to Dr. J. Williamson, for completing treatment in a number of cases and keeping others under observation ; to Dr. G. S. Park Noble, for reporting on cases from January to March, this year.

I have to thank Dr. J. O. Shircore, Director of Medical and Sanitary Services, Tanganyika Territory, for permission to publish this paper.



# THE RODENTS OF LAGOS AND THEIR ECTO-PARASITES WITH REFERENCE TO PLAGUE

BY

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Bubonic Plague first made its appearance in Lagos late in July, 1924. A vigorous 'rat campaign' was at once instituted. Under the supervision of the Medical Officer of Health, several Sanitary Inspectors, with a number of trappers, were detailed for the destruction and collection of rats.

The animals were labelled and taken to a collecting station (equipped by the Medical Research Institute), where their species was determined and their ecto-parasites collected, after which they were dissected and examined.

Four areas were operated, namely Lagos, Iddo, Ebute-Metta and Apapa. All of these are within the Municipal Boundary, the two former being separated from each other and from the two latter by an expanse of water; several miles of swampy land lie between Ebute-Metta and Apapa. Early in 1925, cases of Plague occurred at Agege, a village some twelve miles inland by rail from Ebute-Metta, and rat-trapping was conducted there also.

Cases of Human Plague occurred only in Lagos and Agege. The epi-zoötic was found in the rats only in Lagos, Iddo and Agege. Ebute-Metta and Apapa remained free from the infection.

The methods of rat destruction were trapping and various poisoning devices. The latter were discontinued after a short trial.

The traps used were mainly of the 'break-back' type, and although many attempts were made to lure the animals into cages, these met with but little success. Consequently very few live

rodents were obtained. The rodents dealt with were the black rat, *Rattus rattus*, the brown rat, *R. norvegicus*, the mouse, *Mus musculus*, the shrew, *Crocidura manni*, the striped field-rat, *Lemniscomys fasciatus*, and the pouched rat, *Cricetomys gambianus*.

Plague occurred in the first four named but not in the last two; very few specimens of the last two, however, were collected.

Amongst the black rats there were several more or less marked variations in colouring and, to a less extent, in shape. Some individuals came near *R. r. frugivorus*. In the Agege rats particularly, there was evidence of breeding between different species.

*Mus musculus* greatly predominated in the total catch. They had become so numerous by June, 1925, that it was found impossible to dissect them all. For the first month or two of the campaign, however, in each of the areas, the black rat was caught in greater numbers than any of the other species of rodents. The brown rat was third in numerical importance and fourth place was taken by the shrew.

From the scarcity of *Lemniscomys fasciatus* and *Cricetomys gambianus* in the daily bag, it would appear that these rodents are not usual frequenters of the houses and stores.

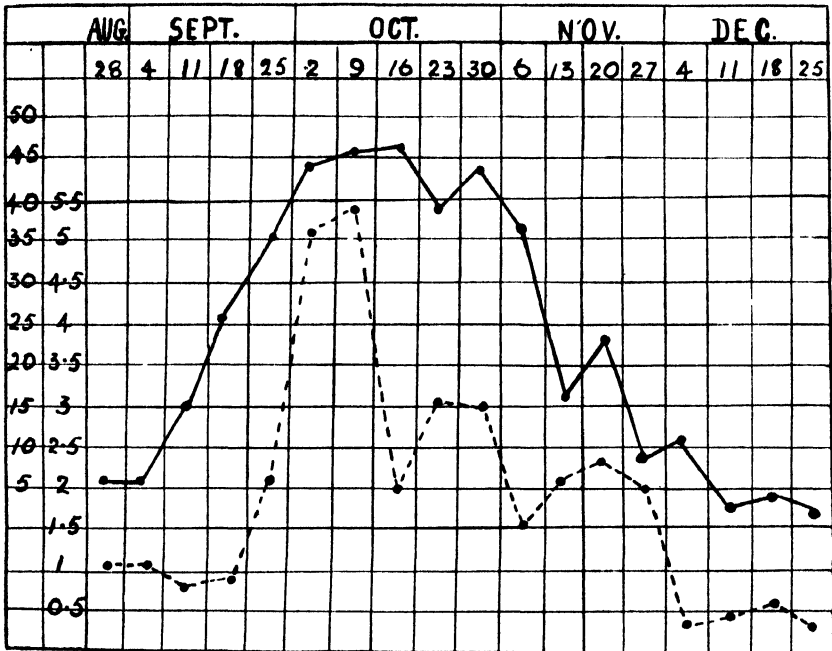
For the detection of plague-stricken animals, two smears were taken from the cut surface of the spleen. The small staff available and the large number of specimens dealt with daily, rendered it impossible to make a complete examination of each individual. As a rule, however, the acute congestion seen on section and the excess of fluid in the serous cavities were an obvious indication of the presence of Plague. Carbol-thionin blue was the principal stain used for the spleen-smears, as it appeared to demonstrate the bi-polarity of the bacillus most clearly. It was observed that, in positive smears, the violet tint of the stain is altered to a slatey-blue. If all the slate-blue smears were picked out they were found to contain practically all the positives, although some showed organisms other than *B. pestis*. In cases of doubt, the duplicate spleen smear was treated with Gram's stain.

As regards plague-infected rodents, it is possible that their number is somewhat under-estimated, for two reasons given above, namely, because many mice were not dissected and because only spleen smears were examined microscopically.

Chart I shows the relationship of human and rat plague during the latter half of 1924. During 1925 this was not so plainly shown by

graphic methods. The Chart indicates that, from 4 September until 11 December, a rise in the percentage of infected rats preceded, by one week, a rise in the number of human cases. Both before and after this period the number of human cases was small.

CHART I.



Dotted lines show percentage of infected rats.

Uninterrupted lines show total human cases of plague per week

28th August—25th December, 1924.

Table I gives the details of the rodents collected in the area of Lagos. August and September have been grouped together because, although trapping was begun in August, the system was not in complete working order until September. The total monthly catches show a steady increase rising from 2,207 in August-September, 1924, to 16,129 in December, 1925. The growing catches were due to greater efficiency as time passed, and are also to be explained by the response from the general public to a definite rate of payment for rats brought to the collecting station. The mouse, except during the first three months, predominated over the black rat and, in the final figures, the proportion is three to one. The black rat out-

numbered the brown rat by nearly eight to one. The shrew was somewhat less common than the brown rat. No specimens of the pouched rat or of the striped rat were obtained.

TABLE I.  
The Rodents of Lagos.

Month	<i>R. ratt.</i>	<i>R. norv.</i>	<i>M. musc.</i>	<i>C. manni.</i>	Total	Infected
1924.						
August-September ...	1,200	488	519	...	2,207	17r, 10n, 6m, 3s
October ...	1,358	125	778	217	2,478	56r, 5n, 15m, 3s, 79
November ...	1,250	65	1,216	314	2,845	42r, 4n, 6m, 3s, 55
December ...	960	54	2,290	295	3,599	13r, 2m, 15
1925.						
January ...	1,042	55	3,816	220	5,133	7r, 5m, 12
February ...	974	96	4,547	243	5,860	1r, 2m, 3
March ...	999	203	4,942	181	6,325	1m, 1
April ...	1,041	95	4,206	158	5,500	0
May ...	1,387	186	6,199	177	7,949	1m, 1
June ...	2,837	320	9,986	261	13,404	9r, 9
July ...	3,405	456	10,956	252	15,069	8r, 8
August...	3,224	473	10,385	203	14,285	5r, 1n, 6
September ...	2,822	535	10,342	131	13,830	35r, 5n, 40
October ...	3,558	454	10,957	174	15,143	65r, 8n, 73
November ...	3,298	461	10,897	120	14,776	52r, 6n, 58
December ...	2,708	442	12,861	118	16,129	65r, 6n, 71

NOTE.—In the last column *r* signifies *R. rattus*.

*n* " *R. norvegicus*.

*m* " *M. musculus*.

*s* " *C. manni*.

TOTALS.—*Rattus rattus* ... 32,063, Infected 375

*Rattus norvegicus* ... 4,508, " 45

*Mus musculus* ... 104,897, " 38

*Crocidura manni* ... 3,064, " 6

144,532, " 464

Of the four species of rodents, there were fluctuations from month to month except in the case of the mice.

As regards plague infection, the black and the brown rat were affected in nearly equal proportion, that is, about 1 per cent.

It is curious to note that the increase in the number of infected rats during the last four months of 1925 co-incided with no great increase in the number of human cases.

TABLE II.  
The Rodents from Iddo

Month	<i>R. ratt.</i>	<i>R. norv.</i>	<i>M. musc.</i>	<i>C. manni.</i>	<i>L. fasc.</i>	<i>C. gamb.</i>	Total	Infected
1924.								
October ...	124	4	55	11	...	...	194	5r, 1s, 6
November	222	13	90	68	...	...	393	12r, 1n, 1s, 14
December	94	2	56	38	...	2	192	5r, 5
1925.								
January ...	115	2	35	76	1	...	229	...
February	99	...	33	35	1	...	168	...
March ...	131	1	43	20	2	..	197	...
April ...	120	...	121	10	.	.	251	..
May ...	111	1	210	5	...	...	327	..
June ...	106	...	179	..	..	...	285	...
July ...	82	..	174	1	...	...	257	...
August ...	231	...	120	7	...	..	358	..
September	80	...	143	2	.	...	225	..
October ...	115	1	110	2	..	...	228	..
November	110	...	94	12	...	...	216	...
December	134	2	162	6	..	...	304	...

NOTE.—In the last column r signifies *R. rattus*,  
n " *R. norvegicus*,  
s " *C. manni*.

TOTALS.— <i>Rattus rattus</i> ...	1,874	Infected 22
<i>Rattus norvegicus</i> ...	26	" 1
<i>Mus musculus</i> ...	1,625	" ...
<i>Crocidura manni</i> ...	293	" 2
<i>Lemniscomys fasciatus</i> ...	4	" ...
<i>Cricetomys gambianus</i> ...	2	" ..
	3,824	" 25

Table II gives the figures of the rodents captured in Iddo. This island is a much smaller one than Lagos, and it yielded a comparatively small catch. Trapping was begun in October, 1924, and the results differed greatly from those seen in Table I. The black



rat, in nine months of the period under review, out-numbered the mouse and the total count for fifteen months shows 1,874 black rats to 1,625 mice. The shrew ranked next to the mouse in numbers and it appeared in the proportion of ten to one of the brown rat. Only twenty-six specimens of the brown rat, four of the striped rat and two of the pouched rat were obtained.

Infected animals were observed in a higher proportion than that found in Lagos during the last three months of 1924, but during 1925 there were no signs of infection.

TABLE III.  
The Rodents from Ebute-Metta.

Month	<i>R. Ratt.</i>	<i>R. norv.</i>	<i>M. musc.</i>	<i>C. manni.</i>	<i>L. fusc.</i>	<i>C. gamb.</i>	Total.
1924.							
November ...	115	4	251	3	...	...	373
December ...	298	10	574	24	...	...	906
1925.							
January ...	350	2	714	57	2	...	1,125
February ...	290	...	915	53	2	...	1,260
March ...	286	...	1,001	42	1	...	1,330
April ...	294	...	1,195	31	1	1	1,522
May ...	343	...	1,337	22	...	1	1,703
June ...	420	...	967	22	...	...	1,409
July ...	452	...	1,164	17	...	...	1,633
August ...	386	1	1,139	23	...	...	1,549
September ...	382	2	1,036	14	...	...	1,434
October ...	306	...	997	4	...	...	1,307
November ...	188	5	791	4	...	...	988
December ...	129	10	615	6	...	...	760

TOTALS.—*Rattus rattus* ... .. 4,239  
*Rattus norvegicus* ... .. 34  
*Mus musculus* ... .. 12,696  
*Cricodura manni* ... .. 322  
*Lemniscomys fasciatus* ... .. 6  
*Cricetomys gambianus* ... .. 2

17,299

Table III deals with the rodents collected at Ebute-Metta. This district ranks next to, but is considerably smaller than Lagos in extent. The proportion of the mouse to the black rat is the same as in Lagos, i.e., three to one. The other figures, however, correspond more closely to those found in Iddo. The shrew is more common than the brown rat in the ratio of ten of the former to one of the latter. Only thirty-six brown rats were obtained and, in addition, there were six striped and two pouched rats.

No infected animals were found.

Tables IV and V concern the rodents from Apapa and from Agege. Both districts are small and the period during which trapping was in progress was short. Both tables show a preponderance of the black rat over the mouse, this being more marked in Agege than in Apapa.

No specimens of the brown rat were obtained in Agege.

TABLE IV  
The Rodents from Agege

Month	<i>R. ratt.</i>	<i>M. musc.</i>	<i>C. mann.</i>	<i>L. fasc.</i>	Total	Infected
1925						
March ... ..	34	11	1	...	46	57, 1m, 6
April ... ..	76	32	1	..	109	12, 1
May ... ..	52	27	2	...	81	..
June ... ..	141	68	...	3	212	..
July ... ..	182	83	2	4	271	...
August ... ..	79	14	4	1	98	...
September ... ..	16	17	1	..	34	...
October... ..	25	11	..	...	36	...

NOTE.—In the last column *r* signifies *R. rattus*.  
*m* " *M. musculus*.

TOTALS.—*Rattus rattus* ... 605, Infected 6  
*Mus musculus* ... 263, .. 1  
*Crocidura mann* ... 11, ..  
*Lemniscomys fasciatus* ... 8, ..  
 887 .. 7

TABLE V.

The Rodents from Apapa.

Month	<i>R. ratt.</i>	<i>R. norv.</i>	<i>M. musc.</i>	<i>C. manni.</i>	Total
August ... 1925. ... ..	49	4	...	12	65
September ... ..	109	7	21	4	141
October... ..	64	5	106	17	192
November " ... ..	40	3	88	4	135
December ... ..	41	6	65	7	119
TOTALS.— <i>Rattus rattus</i> ... ..	...	...	...	...	303
<i>Rattus norvegicus</i> ... ..	...	...	...	...	25
<i>Mus musculus</i> ... ..	...	...	...	...	280
<i>Crocidura manni</i> ... ..	...	...	...	...	44
					652

## ECTO-PARASITES

Four species of ecto-parasites were obtained from four species of rodents (*R. rattus*, *R. norvegicus*, *M. musculus* and *C. manni*). These were *Xenopsylla cheopis*, *X. brasiliensis*, *Ctenocephalus canis* and *Laelaps echidninus*.

As previously noted, very few live rats were received, so that the collection now to be described came mainly from dead animals.

The rodents, as a routine measure, were brought to the laboratory in pails containing a liquid disinfectant. In order to obtain the ecto-parasites, each rat was well shaken in the fluid which was thereafter passed through a sieve of a mesh sufficiently small to prevent the escape of the insects. The retained matter in the sieve was then washed into a white enamelled basin from which the fleas and *Laelaps* could be readily picked out with forceps. This procedure, being somewhat laborious, could not be carried out in Lagos during 1925. The figures which follow, therefore, refer for the most part to Ebute-Metta, Iddo and Agege.

Only twenty-six live rats were obtained. During the last three months of 1924, fifteen *R. rattus* were killed with chloroform and searched. Only six yielded fleas and these numbered sixty-eight, as follows: 15, 14, 14, 12, 9, 4; an average of 11 per rat infested.

In March, 1925, two *R. rattus* were received in a single cage, from which four male *X. cheopis* were obtained. Of three live *R. rattus* caught in September, one had no fleas, one had four fleas and a *Laelaps*, and the third had seventeen fleas. These were identified as *X. cheopis*, 10 ♂♂, 3 ♀♀, *X. brasiliensis*, 4 ♂♂, 4 ♀♀, and *L. echidninus*, 1. In October there were received three *R. norvegicus* in one cage, two *R. rattus* in one cage, and a single *R. rattus* in a cage. The first three animals carried *X. brasiliensis*, 5 ♂♂, 1 ♀, the next two, *X. cheopis*, 2 ♀♀, *L. echidninus*, 3, and the single rat, *X. cheopis*, 1 ♀, 1 ♂.

These findings are included in the following figures which deal with the much larger number of insects recovered from dead rodents.

Tables VI, VII and VIII show the distribution of the ectoparasites in the areas of Lagos, Iddo, Ebute-Metta and Agege. Iddo is included in Table VI for 1924, and in Table VII for 1925.

TABLE VI.  
Ecto-Parasites from Lagos and Iddo.

Month	<i>X. cheopis</i>		<i>X. brasiliensis</i>		<i>C. canis</i>	<i>L. echidninus</i>
	♂	♀	♂	♀	♂	
1924.						
September ... ..	8	5	12	5	1	4
October ... ..	58	41	37	31	...	5
November... ..	156	129	63	45	..	4
December ... ..	69	54	66	39	..	5
1925.						
January ... ..	4	6	8	5	..	14
March ... ..	4	...	...	...	...	..
October ... ..	1	3	...	...	...	3
	300	238	186	125	1	35

TOTALS.—*Xenopsylla cheopis* ... .. 538  
*Xenopsylla brasiliensis* ... .. 311  
*Ctenocephalus canis* ... .. 1  
*Laelaps echidninus* ... .. 35  
885

TABLE VII. Exto-Parasites from Ebute-Metta and Iddo.

Month				<i>X. cheopis</i>		<i>X. brasiliensis</i>		<i>C. canis</i>		<i>L. ecbidinus</i>
				♂	♀	♂	♀	♂	♀	
1924.										
December	...	...	...	13	4	5	5	8	3	...
1925.										
January	...	...	...	16	12	3	1	...	...	10
February	...	...	...	11	13	3	12	...	...	16
March	...	...	...	26	17	8	7	...	...	3
April	...	...	...	17	15	7	7	...	...	3
May	...	...	...	11	21	7	12	...	...	19
June	...	...	...	23	18	12	6	...	...	5
July	...	...	...	26	9	10	3	...	...	31
August	...	...	...	1	1	3	1	...	...	5
September	...	...	...	25	7	8	13	...	...	4
October	...	...	...	5	3	13	1	...	...	8
				174	120	79	68	8	3	104

TOTALS.—	<i>Xenopsylla cheopis</i>	...	...	...	294
	<i>Xenopsylla brasiliensis</i>	...	...	...	147
	<i>Ctenocephalus canis</i>	...	...	...	11
	<i>Laelaps ecbidinus</i>	...	...	...	104
					556

TABLE VIII. Ecto-Parasites from Agege.

Month				<i>X. cheopis</i>		<i>X. brasiliensis</i>	<i>L. ecbidinus</i>
				♂	♀		
March, 1925	...	...	...	2	2	...	3
April	...	...	...	7	2	1	10
May	...	...	...	...	...	...	...
June...	...	...	...	...	...	...	2
July	...	...	...	...	2	...	1
				9	6	1	16

TOTALS.—	<i>Xenopsylla cheopis</i>	...	...	...	15
	<i>Xenopsylla brasiliensis</i>	...	...	...	1
	<i>Laelaps ecbidinus</i>	...	...	...	16

It has not been possible to collect data regarding variations in the number of fleas or in the preponderance of one species over another at different seasons. Many, if not most of the insects, must have left their host between the time of its death in the trap and its collection by the trapper. Nevertheless, as over 1,000 fleas have been examined, it is probably correct to assume that *X. cheopis* is more common than *X. brasiliensis*, the actual numbers identified being 847 of the former, to 459 of the latter. In all the three species found, the male predominates over the female.

Some interesting experiments were done early in 1925, using guinea-pigs as flea-traps. The results of these experiments did not confirm the above finding, that *X. cheopis* is more common than *X. brasiliensis*.

On 8th January, two guinea-pigs were allowed to run loose in a room from which a Plague corpse had been removed a few hours earlier. This room had not been disinfected. After two minutes the guinea-pigs were re-captured, put into a white cloth sack and taken back to the laboratory where they were lightly chloroformed. Twenty-one fleas were recovered from them, *X. brasiliensis*, 19, and *X. cheopis*, 2.

On 17th January the experiment was repeated but on this occasion the room had been disinfected twenty-four hours previously. The guinea-pigs got in between a double row of palm mid-ribs which formed one of the walls of the house, and twenty minutes elapsed before they were re-captured. They were found to have collected thirty-three fleas, *X. brasiliensis*, 28, and *X. cheopis*, 5.

On the same day, two guinea-pigs were allowed to run loose in a non-infected house for two minutes. These collected no fleas.

On 19th January the experiment was repeated in a room which had been disinfected forty-eight hours previously. Two fleas were recovered, both *X. cheopis*.

Disinfection in the above instances had been by means of burning sulphur in the approved manner.

In the final experiment, on 12th February, the room had been disinfected twenty-four hours previously by means of spraying with a mixture of kerosene and cyllin. No fleas were found on the two guinea-pigs which had had two minutes' freedom in this room.

### SUMMARY

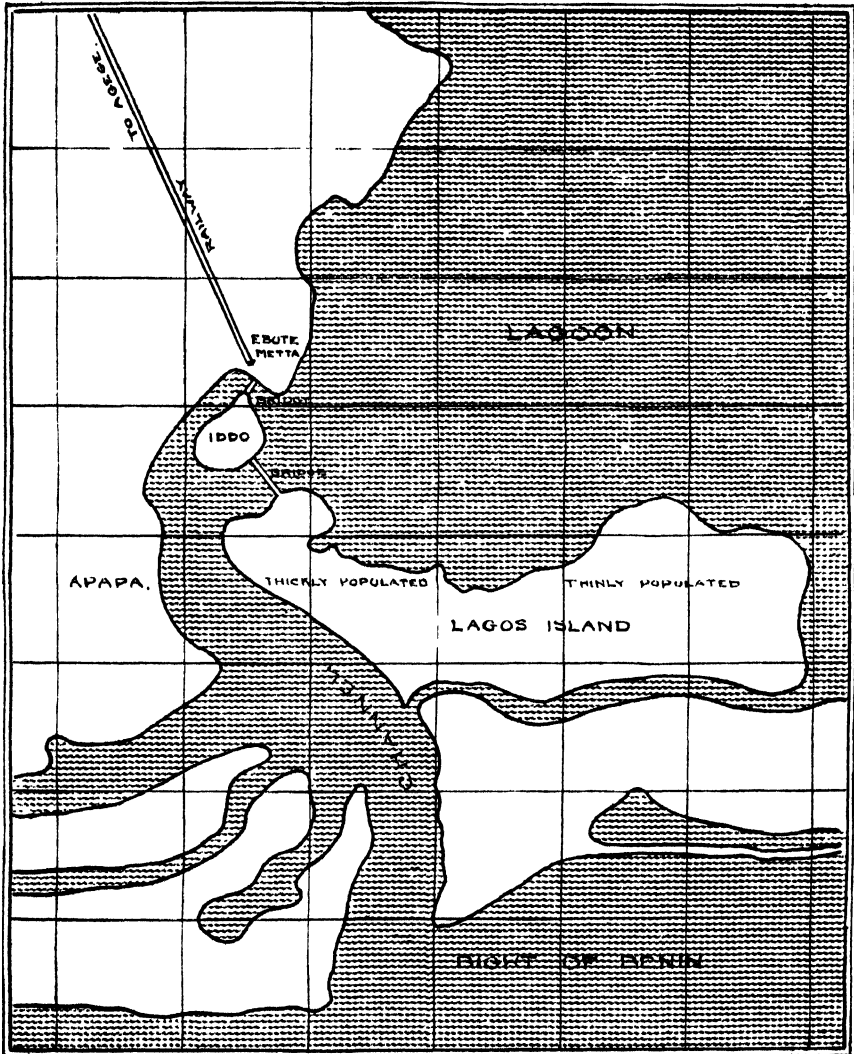
One hundred and sixty-seven thousand, one hundred and ninety-four rodents are dealt with, along with one thousand, five hundred and twenty-nine ecto-parasites.

The area investigated is Lagos and its environs.

The rodents found are *Mus musculus*, *Rattus rattus*, *Rattus norvegicus*, *Crocidura manni*, *Lemniscomys fasciatus* and *Cricetomys gambianus*. The ecto-parasites are *Xenopsylla cheopis*, *Xenopsylla brasiliensis*, *Laelaps echidninus* and *Ctenocephalus canis*.

### ACKNOWLEDGMENTS

Dr. Andrew Crawford, M.B.E., and Dr. Naudi superintended the arrangements for rat-trapping and helped very greatly in the investigations. Mr. E. F. Hines and Corporal Bowrey, R.A.M.C., rendered invaluable assistance in the numbering and the dissection of the rodents. Mrs. Summers Connal, M.B.E., is responsible for the identification of the ecto-parasites.







THE IDENTITY OF  
*LEISHMANIA TROPICA* WRIGHT, 1903,  
AND *HERPETOMONAS PAPATASII*  
ADLER, 1925

BY

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(*Received for publication 10 September, 1926*)

PLATES XXVIII, XXIX

The authors (1925 and 1926) have recorded three cases of experimental transmission of cutaneous Leishmaniasis to man from naturally infected specimens of *Phlebotomus papatasi* ♀♀. These experiments in themselves are not sufficient to prove that the sand-fly is a natural carrier of cutaneous Leishmaniasis, for since Laveran and Franchini (1913 and 1916) infected mice with *Herpetomonas* from various insects, Fantham and Porter (1914 and 1915) infected animals with *Herpetomonas* from species as widely removed from each other as *Nepa cinerea* and *Ctenocephalus canis*, and Fantham (1922) produced Leishmaniasis in a young rat by injecting it with *Herpetomonas muscae domesticae*, it can be argued that any *Herpetomonas*, whatever its source, if introduced into the human skin, may give rise to cutaneous Leishmaniasis. It is true that Hoare (1921), Becker (1923), Shortt (1923) and Drbohlav (1925), among others, have repeated the experiments of Laveran and Franchini and of Fantham and Porter, with negative results but, as Becker has pointed out, these results 'can have such value only as is ordinarily attributable to negative results.'

There is one point of particular importance for the interpretation of negative results in experiments with *Herpetomonas* from insects on man or animals which has been overlooked by all writers on the subject, i.e., *Herpetomonas* sp. in insects are polymorphic and not

all the forms are necessarily infective. The *Leishmania* forms and many of the flagellate forms in artificially infected *P. papatasi* are not infective for man as the experiments of Adler and Theodor (1926) conclusively prove.\*

Evidence is accumulating to the effect that the development of *Leishmania tropica* in *Phlebotomus papatasi* is a biological one with infective forms as the end point of the cycle in the sandfly as predicted by Adler (1925). Infective forms need not necessarily be present in an insect containing *Herpetomonas* and this may explain some of the negative results obtained in experiments with *Herpetomonas* on animals. For the same reason the *uniformly* positive results of Fantham and Porter are difficult to understand.

Noguchi (1924) and Kligler (1925 and 1926) independently introduced agglutination methods which demonstrate the specificity of the three species of *Leishmania* of man, methods which promise to simplify the whole problem of experimental Leishmaniasis and liberate it from the conflicting evidence so far obtained from the injections of *Herpetomonas* from various insects into animals. Agglutination methods promise to be of great value not only in Leishmaniasis but also in the study of cultivable *Herpetomonas* in general. Hitherto it has been customary to name every *Herpetomonas* found in a new host as a new species, for differentiation of species on morphological grounds is within wide limits obviously impossible.

Becker (1923) and Drbohlav (1925) have successfully carried out cross-infection experiments with *Herpetomonas* from various flies and on the basis of these cross-infection experiments Becker considers it extremely probable that *Herpetomonas muscae domesticae*, *H. luciliae*, *H. calliphorinae* and *H. sarcophagae* are one species and Drbohlav considers the *Herpetomonas* of *Lucilia serricata*, *L. cesarea*, *Fannia regina* and *Musca domestica* as identical. It must be pointed out that cross-infection experiments in insects and in general artificial inoculation of insects with *Herpetomonas*, though suggestive, can never in themselves be conclusive and may actually prove misleading,

\* Sergeant, Edm. and Et., Parrot, L., Donatien, H., and Réguet, M. (1926) have recently republished, with many additional details, their experiment of 1921 in which they produced oriental sore in man by inoculation of an infusion of seven sandflies. In the original paper (1921) they state, 'Le liquide de broyage, examiné au microscope, ne montre ni flagellés ni aucune autre forme parasitaire,' and they repeat this statement in their recent paper. As it is inconceivable that the result obtained should have been produced in the absence of *Herpetomonas* we are convinced that *Herpetomonas* were present but were overlooked.

e.g., the bed bug which is a natural host of neither *Herpetomonas donovani* nor *H. tropica* can be artificially infected with both these flagellates. On the other hand one species of flagellate may be inoculable into a various number of arthropod hosts, e.g., *Schizotrypanum cruzi* is infective for *Conorrhinus megistus*, *Cimex lectularius* and *Ornithodoros moubata*. It thus appears certain that the method of the future for determining *Herpetomonas* sp. will be a serological one. Such a method, if successful, will greatly facilitate investigations on oriental sore and Kala-azar for if an insect is suspected of being a carrier of these diseases, a comparison of its naturally occurring *Herpetomonas* with cultures of *Leishmania donovani* and *L. tropica* by Noguchi's or Kligler's method will quickly confirm or dispel this suspicion. Serological methods should also prove decisive in settling the long-standing problem of the relationship between human and canine Leishmaniasis in localities where these two diseases are prevalent.

The three experimental strains were compared by cross agglutinations with three strains from naturally acquired oriental sores.

### EXPERIMENTAL STRAINS

STRAIN I. The lesion in this case was a papule. By April 26th the papule was 3 mm. in diameter, Leishman-Donovan bodies which had hitherto been few became numerous and cultures were readily obtained.

STRAIN II. The lesion consisted of a hard subcutaneous nodule.

STRAIN III. The lesion consisted of two ulcers.

The above lesions and the details of their transmission and development were described in a previous communication. In the case of Strain III the two lesions healed spontaneously by July, 1926, i.e., about eight months after inoculation.

STRAIN IV. This strain was isolated by Professor I. J. Kligler, from an oriental sore acquired in Baghdad.

STRAIN V. Isolated by Professor I. J. Kligler, from a case from Artuf (Palestine).

STRAIN VI. Isolated by Dr. R. Junovitch, of the Rothschild Hospital, from a case acquired in Jerusalem.

All the strains were cultured on the following modification of Noguchi's leptospira medium :

Agar, 1 part.

Locke's solution containing 0.2 % dextrose, 8 parts.

Fresh rabbits' serum, 1 part.

On the above medium growth of *L. tropica* is fairly rapid, a uniform thick growth being produced in the upper 4 mm. of the tube five to seven days after inoculation.

The above medium was also found to be very satisfactory for the culture of *Schizotrypanum cruzi*.

Noguchi (1924) showed that if his leptospira medium is made up with immune rabbit serum instead of normal serum, the flagellates, instead of growing in a uniform layer at the top of the medium, grow in clumps for a varying distance throughout the medium ; also, if immune serum is added to normal cultures, these cultures become agglutinated. This reaction Noguchi showed to be specific for each of the three human species. We employed both methods for comparing the various strains. Immune serum for each strain was prepared by four intravenous injections into rabbits of pure culture at four days' interval, the first injection being 0.5 c.cs. and the fourth 2.0 c.cs. ; each rabbit received a total of 5 c.cs. pure culture.

With Noguchi's method the results were quite decisive and the six strains were found to be identical.

The growth in media made up with immune serum consists either of globoid masses (Pl. XXVIII, figs. 2-7) scattered through the medium, or globoid masses together with irregular granular flakes of various size (Pl. XXVIII, figs. 2-7). Each globoid mass consists of a homogeneous mass of Leishman-Donovan bodies (Pl. XXIX, figs. 1-4) and may or may not contain at its periphery a number of forms with rudimentary flagella. The irregular flakes consist of masses of Leishman-Donovan bodies and flagellates in varying proportion (Pl. XXIX, figs. 5-6).

The specific serum produces a far profounder effect on the organisms than mere agglutination for if subcultures on medium made up with normal serum are made from cultures grown on medium made up with 10 per cent. specific serum, growth takes place not in the upper three or four millimetres, as in a normal culture,

but from the surface down to a depth of several centimetres (Pl. XXVIII, figs. 8-9), a phenomenon noted by Noguchi to occur in normal cultures of *Leishmania donovani*.

This reaction, which shows a change of oxygen requirement on the part of the organism, is not due to agglutinin for the amount of serum picked up in a single loopful of culture is not sufficient to alter a normal culture. This change in oxygen requirement persists in successive subcultures for two to five generations.

Kligler's method consists of agglutination in test-tubes with various dilutions of serum. Its chief drawback lies in the difficulty of interpretation due to the tendency of *Leishmania tropica* in culture to form rosettes, and in the time required to make a suspension of *Herpetomonas* free from media. The following technique was used. One part pure culture is mixed with six parts saline and centrifuged; the supernatant fluid is removed and replaced by an equal quantity of saline, the mixture is shaken and again centrifuged. This is repeated at least three times and finally a suspension is made of one part original pure culture to six parts saline. (The above method has been used by one of us (A.) during the last six months for the preparation of vaccine.) The suspension is divided into tubes and the agglutination is performed in the usual way. It is absolutely essential that the cultures used for the agglutination should not be recently isolated but should be at least five or six generations old, otherwise the suspension tends to contain granular masses of parasites and is useless for agglutination. Using the above method an immune serum gives a titre of up to 500.

The tubes are read after twenty-four hours. The following titres were obtained :

Serum 1, 100.

Serum 2, 200.

Serum 3, 500.

Serum 4, 200.

Serum 5, 100.

Serum 6, 200.

After reading, the tubes should be shaken and it will be noted that in the positive tubes a uniform suspension cannot be made, for granular masses of various sizes are distributed through the suspension. These masses on microscopic examination are found to be clumps of agglutinated organisms. If the control tubes contain

a deposit, which sometimes happens in spite of all care, a uniform suspension is formed on shaking. In lower dilutions (1 to 40) there is no difficulty in reading the results.

The above method cannot be considered final and much work still requires to be done in producing a technique of agglutination in tubes for *Herpetomonas* which will lend itself to standardisation.

It is interesting to note that Napier's formaldehyde test was negative in the three experimental cases and in the six immunised rabbits. During 1924 and 1925 one of us (A.) examined the sera of thirteen naturally infected cases of oriental sore from Dr. A. Dostrowsky's clinic for this reaction, and all were negative.

The serological tests prove that the parasite of all the three experimental lesions produced by the inoculation of *Herpetomonas* from naturally infected *Phlebotomus papatasi* ♀ into man was no other than *Leishmania tropica*, in other words, *Herpetomonas papatasi* is a synonym of *Leishmania tropica* and *Leishmania tropica* is a natural parasite of *Phlebotomus papatasi*.

The number of clinical varieties of oriental sore has given rise to the suspicion that there are varieties of *Leishmania tropica* and Brumpt has gone so far as to create a species *Leishmania nilotica* Brumpt, 1913, for the parasite of a nodular form of cutaneous Leishmaniasis occurring in the Sudan. The above serological observations show that three experimental lesions which clinically differed so markedly were caused by the same parasite and the correctness of the serological interpretation is proved by the following experiments.

EXPERIMENT I, 20.4.26. Volunteer inoculated (intracutaneously) on two points on left forearm direct from Case II (Nodule). 16.6.26. A papule was noted on one of the inoculated points and, on examination, Leishman-Donovan bodies were found.

EXPERIMENT II, 20.4.26. A volunteer was inoculated into two points on the left forearm direct from Case II. The skin was incised and the inoculation was made with a capillary into the subcutaneous tissue. No lesion has yet appeared (after four months).

The result of Experiment No. I proves that there is no relationship between a strain of *Leishmania tropica* and the clinical type of lesion produced in man which therefore depends entirely on factors present in the infected individual. We have to thank Professor I. J. Kligler and Dr. R. Junovitch for the gift of strains of *Leishmania tropica*.

## CONCLUSIONS

*Herpetomonas papatasi* is a synonym of *Leishmania tropica*. *Leishmania tropica* is a natural parasite of *Phlebotomus papatasi* and the proof that the latter is a natural carrier of cutaneous Leishmaniasis is complete.

There is no relationship between a strain of *Leishmania tropica* and the clinical type of cutaneous Leishmaniasis produced in man.

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## PLATE XXVIII

Fig. 1. Normal culture.

Figs. 2-7. Show types of agglutination in culture on media made up with immune serum. Note in each tube individual globoid masses and diffuse growth in varying proportions.

Fig. 2. Strain I grown on serum 2.

Fig. 3. Strain II grown on serum 2.

Fig. 4. Strain IV grown on serum 1.

Fig. 5. Strain III grown on serum 4.

Fig. 6. Strain IV grown on serum 2.

Fig. 7. Strain V grown on serum 4.

Figs. 8-9. Subcultures on media made up with normal serum from strains grown on immune serum. Note deep growth.

Fig. 8. Strain V.

Fig. 9. Strain II.

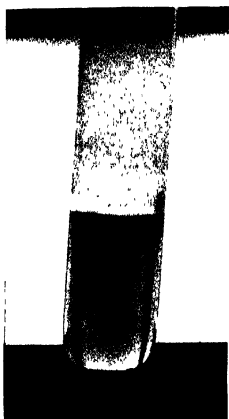


FIG. 1



FIG. 2



FIG. 3



FIG. 4



FIG. 5



FIG. 6



FIG. 7



FIG. 8



FIG. 9





## PLATE XXIX

- Figs. 1-2. Colonies consisting entirely of Leishman-Donovan bodies from culture made up with immune serum.  $\times 65$ .
- Figs. 3-4. Same type of colonies.  $\times 250$ .
- Figs. 5-6. Masses consisting of Leishman-Donovan bodies and flagellates from diffuse growth in immune serum.  $\times 250$ .
- Figs. 7-8. Fields from a normal culture. Fig. 7.  $\times 250$ . Fig. 8.  $\times 200$ .



FIG. 1

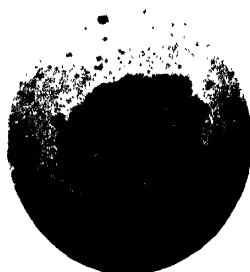


FIG. 2

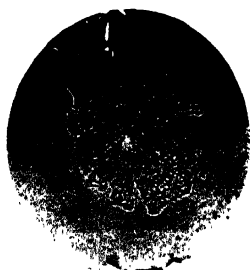


FIG. 3



FIG. 4



FIG. 5

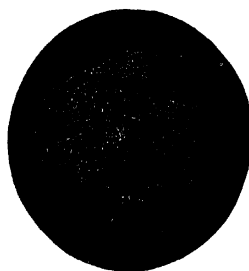


FIG. 6

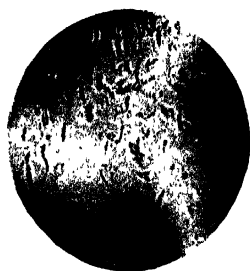


FIG. 7

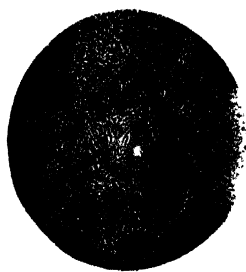


FIG. 8



# YAWS

## RESULTS OF NEOSALVARSAN THERAPY AFTER FIVE YEARS

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At the request of the Military Government of the Dominican Republic the School of Tropical Medicine, Harvard University, sent a commission consisting of Drs. A. W. Sellards, W. L. Moss, and G. H. Bigelow to Santo Domingo during the summer of 1920, to study yaws. The results of the observations made were published in the *John Hopkins Hospital Bulletin* in February, 1922. During the summer of 1925, the writer returned to Santo Domingo for the purpose of observing the results of neosalvarsan therapy after an interval of five years, and collecting such additional data as might be available or of interest in connection with the series of 1,046 cases on which fairly complete records were made in 1920.

To make intelligible this report and for the purpose of comparison, a brief review of the results of the 1920 expedition is necessary.

*Framboesia tropica*, Yaws, or, as it is called in Santo Domingo, 'Buba' is a specific infectious disease of the tropics, caused by the *Treponema pertenue* (Castellani), characterised by a framboesiform granulomatous eruption.

The course of the disease, like that of syphilis, is usually divided into a primary, secondary, and tertiary stage, but we agree with the majority of authors that yaws and syphilis are separate and distinct diseases, and in our previous report produced evidence which seems adequate to prove the correctness of this view.



It is convenient to make several subdivisions of the secondary stage, especially on account of the large number of patients in Santo Domingo who suffer from the condition of the soles of the feet which they call 'clavus.' Patients with this condition as their only active manifestation of yaws, at the time of observation, made up the largest single group in the series studied, and with the exception of the lesions to which they apply the term 'gomma' (tertiary stage), it produces more pain and disability than any other lesions of the disease.

Our view that clavus is a manifestation of yaws having been called into question, it seems necessary to give the evidence on which we base this view ; this is especially necessary since, as stated above, patients with this condition as their only active manifestation of yaws at the time of observation made up the largest single group in the series studied, 327 cases or 31.2 per cent. Every one of these individuals gave a history of having had the primary lesion and having gone through the florid secondary eruption of granulomata elsewhere on the body, and a great majority of them still bore the typical scars of the preceding lesions. Moreover there were 252 patients, comprising 24 per cent. of the entire series, who presented, in addition to clavus, other well recognised lesions of yaws. The above facts, though highly suggestive, do not exclude the possibility that clavus may be a concomitant, though unrelated, condition. More convincing is the observation of every stage in the development of clavus from typical fresh granulomata on the soles of the feet which later undergo the regressive changes which take place in those located elsewhere on the body, except as modified by the difference in the nature of the surface on which they occur. As the granulomata shrink they separate at the margins from the thick plantar surface and become circumvallate, forming a dry hard core which is the 'nail' from which the condition takes its name ; later the core falls out, leaving a 'nail hole,' a circular 'punched out' opening 0.75 to 1 cm. in diameter with sharp cut edges descending vertically 2 or 3 mm. to a clean flat base ; subsequently, aided perhaps by walking on the bare feet, erosion takes place about the 'nail holes' which extends to meet similar erosions about other holes and gives rise to the peculiar moth-eaten appearance of the soles which is characteristic of the majority of these cases in their later stages. These observations

leave no doubt in our minds that clavus is a manifestation of yaws and that a very great majority of patients, in Santo Domingo at least, develop this condition.

It is convenient to use the same abbreviations as were used in our previous report to designate the various lesions and stages of the disease, and if the reader will take a few moments to familiarise himself with these the understanding of this report will be much facilitated :

- M. Madre buba, mother yaw, or primary lesion.
- B'. Florid secondary eruption of granulomata. Early secondary stage.
- B. Sparse recurring secondary granulomata. Late secondary stage.
- C. Late lesions on the soles. Late secondary stage.
- P. Palmer lesions. Late lesions on the palms. Late secondary stage.
- S.L.. Studded Lesions. Late secondary stage (?).
- G. 'Gomma.' Tertiary stage.
- H. History of Yaws. No active lesions present. Quiescent stage.

The term 'studded lesions' was introduced by us to describe a condition which we believe to be a late secondary manifestation of yaws, consisting of moderately hard skin nodules, about 1 cm. in diameter, elevated 3 to 4 mm., not painful, unaccompanied by itching and without striking pigmentary changes until after regression, when an increase of pigmentation may mark their former site. These nodules are thickly studded and regularly set over an area which at first may be not over 8 to 10 cm. in diameter. The size of the area involved may increase to 15 or 20 cm. in diameter by an advancing margin consisting of an almost unbroken row of nodules. As this peripheral advance occurs healing takes place in the centre of the area. In the absence of ulceration retrogression is accompanied by desquamation of epithelium, the nodules gradually flatten out and disappear, leaving no trace, or more often pigmentary changes mark their former site. In no case were the nodules observed to pass through a vesicular stage, but they frequently undergo ulcerative changes probably due to secondary infection.

As pointed out in our previous publication there is much overlapping of the various stages of yaws, an overlapping which far exceeds any which we have observed in syphilis. We think, however, that a very fair idea of the usual sequence of events in yaws may be obtained by arranging the patients observed in 1920 who presented but a single manifestation of the disease (scars excluded) according

to the average age of each group at the time of observation, and comparing this with the average duration of the disease at the same time. This is shown in the following table :

Diagnosis	No. of cases	Average age	Average duration
M	15	6.6 years	2.3 mos
B'	82	8.8 "	12.3 "
B	60	9.3 "	29.6 "
C	327	21.6 "	9 years
H	127	23.8 "	9.7 "
P	5	25 "	14 "
S.L.	37	28.8 "	13.5 "
G	67	29.3 "	16.3 "
	720		

This series comprises 720 cases and the number included in a majority of the groups is large enough to give averages that are probably fairly reliable. This arrangement according to average ages probably indicates the usual sequence in which the various lesions develop. Confirmatory evidence of the correctness of this view is furnished by the fact that the average duration of the disease, at the time of observation in the various groups, with one exception (studded lesions) follows the same orderly progression as do the ages.

The overlapping of the various stages of yaws is well illustrated by a brief recapitulation of some of the tables previously published.

Diagnosis	Number of cases	Diagnosis	Number of cases
M	15	BCP	13
MB'	25	CP	50
B'	82	P	5
MB	6	MG	1
B	60	BG	3
MB'C	13	BCG	2
B'C	24	CG	5
BC	106	CPG	1
C	327	G	67
C+	41	H	55
MBC	5	H+	72

In the above table a plus sign following an abbreviation indicates that there was, in addition to the active manifestation of yaws or the

history of yaws, some other pathological condition present which, in our opinion, was not due to the yaws infection. In a majority of these cases these lesions consisted of old leg ulcers and occurred in the older patients.

Since the observations in 1925 were made largely to determine the late results of the treatment given in 1920 it is necessary, for the purpose of comparison, to review briefly the results of treatment as observed in 1920.

Neosalvarsan was used in the treatment of these cases. The drug was dissolved in freshly distilled water in the proportion of 0.1 gm. to 2 c.c. and injected within thirty to forty-five minutes after being put in solution. The intravenous method was used in all cases except for young children with veins difficult of access, these patients receiving the injections intramuscularly in the buttock. The dose varied from 0.075 gm. for an infant under 1 year of age to 0.6 gm. for a fully developed adult. Intermediate doses were given in proportion to age and body weight. The interval between injections was one week in the majority of cases ; exceptionally it was as short as five days and in a moderate number of cases it was ten days to two weeks.

The results were designated as 'cured,' 'practically cured,' 'much improved,' 'improved,' and 'unimproved.' No case was recorded as 'cured,' unless all the lesions (scars excepted) had entirely disappeared. If there remained at the last opportunity of observation only so much as a few black crusts almost ready to fall, where a week or two before there had been an abundant crop of fresh granulomata, such cases were designated as 'practically cured' although we were convinced that could we have seen these patients a week later, without further treatment they would have fallen into the 'cured' list.

Of course it should be understood that the terms 'cured' and 'practically cured' were not intended to express a judgment as to the final result of therapy so recently given but as indicating the disappearance of lesions and freedom from all symptoms of the disease.

In considering the dosage of neosalvarsan that may have produced a given result it seemed better to express it in terms of the number of injections given rather than in absolute amounts of the drug. The reason for this was that the patients varied widely in age and weight and the effort was made to keep the dosage proportional to age and weight.

The following table, taken from our previous report, gives the results as judged in 1920.

Diagnosis	Number of cases	Result		Cured				Practically cured				Much improved				Improved				Unimproved		
		No Note	Noted	Number of injections				Number of injections				Number of injections				Number of injections				Number of injections		
				1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3
M	15	4	11	2	...	...	...	5	2	1	...	1	...	...	...	...	...	...	...	...	...	...
MB'	25	10	15	2	2	...	...	4	1	...	...	6	...	...	...	...	...	...	...	...	...	...
B'	82	16	66	13	11	1	...	14	6	1	...	8	3	2	...	5	1	...	...	1	...	...
MB	6	3	3	...	...	...	...	...	...	...	...	1	...	1	...	1	...	...	...	...	...	...
B	60	24	36	4	9	2	...	1	3	...	...	11	3	...	...	3	...	...	...	...	...	...
MB'C	13	2	11	...	3	...	...	2	3	...	...	2	...	...	...	1	...	...	...	...	...	...
MBC	5	3	2	...	1	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...
B'C	24	4	20	2	4	...	...	5	5	...	...	3	1	...	...	...	...	...	...	...	...	...
BC	106	26	80	...	10	1	...	7	6	...	...	36	7	1	...	7	3	1	...	1	...	...
C	327	143	184	5	5	5	1	3	9	...	...	27	16	...	...	55	10	...	...	41	7	...
C+	41	13	28	...	1	1	...	1	...	...	...	7	3	3	...	4	2	...	...	6	...	...
BCP	13	7	6	...	3	...	...	...	...	...	...	3	...	...	...	...	...	...	...	...	...	...
CP	50	15	35	...	...	1	...	...	1	1	...	5	7	...	...	10	1	...	...	9	...	...
P	5	3	2	...	...	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...
SL	37	17	20	1	2	1	...	1	...	1	...	3	2	...	...	4	2	...	...	3	...	...
G	67	24	43	...	...	...	...	...	...	...	...	4	5	2	8	...	5	...	...	15	3	1
G+	12	4	8	...	...	...	...	...	...	...	...	4	1	...	...	1	...	...	...	2	...	...
	888	318	570	29	51	12	1	43	36	4	...	113	51	13	2	99	21	6	...	78	10	1
				93				83				179				126				89		

The above table gives the results of treatment in the various groups, as noted at the last observation, on each case and shows the number of injections each patient had received at the time of this note. The 127 patients who gave a history of having had yaws but who had no active lesions of the disease at the time they presented themselves for treatment are not included in this table, as we had no means of judging the results in these cases

at that time. There were also excluded from the table thirty-one cases of yaws in which the results of treatment were difficult to determine owing to complicating diseases or for other reasons. Exclusive of these two groups there were 888 cases and on 570 of these there were notes as to the results of treatment.

Owing to the subdivision of these cases into groups according to the several lesions or stages of the disease which they presented, and the smallness of some of these groups, no attempt at detailed analysis of this table seems worth while. Briefly summarised, irrespective of the number of injections each patient received, this table shows the following results :

	Cases	Per cent.
Cured .. ...	93	16.32
Practically cured .. ...	83	14.56
Much improved .. ...	179	31.41
Improved .. ...	126	22.15
Unimproved .. ...	89	15.61
Total ... ..	57	100.00

The results according to the number of injections, irrespective of the stage of the disease, are shown in the following tabulation

Results	1 Injection, 362 cases		2 Injections, 169 cases		3 Injections, 36 cases		4 Injections, 3 cases	
	Cases	Per cent.	Cases	Per cent.	Cases	Per cent.	Cases	Per cent.
Cured ... ..	29	8.0	51	30.2	12	33.3	1	33.3
Practically cured ... ..	43	11.8	36	21.3	4	11.1	..	..
Much improved ... ..	113	31.4	51	30.2	13	36.1	2	66.7
Improved ... ..	99	27.3	21	12.4	6	16.6	..	..
Unimproved ... ..	78	21.5	10	5.9	1	2.9	..	..
	362	100.0	169	100.0	36	100.0	3	100.0

These results are very disappointing if one accepts at par the claim which some authors have made, that 90 per cent. of yaws cases are cured by a single injection of salvarsan. It is true that in the above series the final observation was made too soon after the administration of the drug to feel sure that the full effect had manifested itself but, even if the cases recorded as 'cured' are combined with those listed as 'practically cured,' the percentage thus obtained (19.8 per cent.) falls far short of the optimistic claim of 90 per cent. cures after one injection.

Of the 169 patients who received two injections 30.2 per cent. were recorded as 'cured' and 21.3 as 'practically cured.' If we

combine these two groups and consider both as 'cured' the percentage so obtained is 51.5.

The numbers of patients receiving three and four injections are so small that it seems wiser not to attempt to draw conclusions from them.

Admitting that these results are disappointing as measured by the extravagant claims of some authors, we have never seen anything more dramatic in the field of therapy than the prompt disappearance of the loathsome lesions and disabling symptoms of yaws after one or two injections of neosalvarsan.

We have further analysed our 1920 figures to see if they will show what stage of the disease is most readily amenable to treatment. For this purpose it seems probable that more reliable information will be obtained by combining the 'cured' and 'practically cured' cases and considering only those who received a single injection and in whom there was no overlapping of the stages of yaws.

Presented in this way the cases are shown in the following table :

Diagnosis	Total number of cases	Average age in years	No. of cases result noted	Cured or practically cured after 1 injection	
M	15	6.6	11	7	63.63
B'	82	8.8	66	27	40.91
B	60	9.3	36	5	13.88
C	327	21.6	184	8	4.34
P	5	2.5	2	0	...
S.L.	37	28.8	20	2	10.0
G	67	29.3	43	0	...

The number of cases comprising some of the groups in the above table is too small to be of value for statistical purposes, but taken as a whole the figures seem to show what we would expect, i.e., the earlier the stage of the disease, the more readily does it respond to treatment.

Of the 1,046 cases studied in 1920 we had the opportunity, in 1925, of actually examining 366 and of getting what appeared to be reliable histories of 53 more. Among the latter group there were a number of patients who had died during the epidemics of dysentery and small-pox which visited this section since 1920. Some had died as the result of other diseases or of accidents ; others in this group whom we did not actually see and examine sent word by adult members of their families that they were 'cured' and could see no reason for undertaking a long tedious journey on foot or by horse simply to report that they had remained free from signs and symptoms of yaws for five years. In no instance did we include a case unless the history seemed worthy of credence.

We are, therefore, able to report on 419 of the original 1,046 cases, an interval of five years having elapsed between the observations.

Some of the more important data on these cases are summarised in the following table :

Diagnosis	No. of cases			Age in years			Duration in years			No. of injections prior last observation				Results in 1920				Free interval, uncured cases		
	M.	F.	Total	Min.	Max.	Av.	Min.	Max.	Av.	1	2	3	4	unob	uc	pc	c	Min.	Max.	Av.
M	4	1	5	4	18	9'4	1/12	6 7/12	3/12	3	2	..	..	...	2	2	1	2 7/12	9/12	11/24
MB'	5	7	12	2	30	10'7	1/12	1	7/12	7	3	...	..	2	3	4	3	1 1/12	4 5/12	2 7/12
B'	22	14	36	1 6/12	30	8'8	2 7/12	2	9/12	18	14	1	..	3	8	13	12	3 1/12	4 11/12	2 10/12
MB	2	...	2	5	35	20'0	1/12	4 1/12	5 2/24	1	...	1	...	..	2	..	...	...	...	...
B	11	12	23	1	81	12'3	4/12	17	2 4/12	7	7	1	...	8	6	2	7	7 1/12	4 9/12	3 1/12
MB'C	4	2	6	1	23	11'6	3 1/12	10 1/12	6/12	3	2	...	...	1	2	2	1	4	4 6/12	4 3/12
B'C	5	4	9	2 6/12	25	14'0	2 1/12	6 1/12	8/12	2	6	..	..	1	1	4	3	2	4 11/12	3 7/12
MBC	1	...	1	...	...	8'0	...	...	7 1/12	...	1	...	...	..	..	1	..	...	...	4
BC	32	15	47	3	35	15'9	6/12	25	5 6/12	23	14	...	...	10	27	6	4	2 1/12	4 11/12	2 4/12
C	101	48	149	3	80	23'0	4/12	55	9	65	26	4	1	53	80	6	10	1 1/12	4 11/12	2 10/12
C+	17	4	21	10	60	28'5	1	16	7 11/12	9	3	3	...	6	13	..	2	1 1/12	4 5/12	1 11/12
II	14	16	30	2	50	20'3	6/12	27	6 10/12	25	4	1	...	...	...	...	1 1/12	4 11/12	2 7/12	
II+	4	4	8	2	39	19'0	6/12	34	8 10/12	5	2	1	..	...	...	...	4 9/12	4 10/12	4 10/24	
MB'CP	1	...	1	..	..	15'0	...	...	1 1/12	1	...	...	...	...	...	1	...	...	...	3
BP	...	2	2	20	30	25'0	6	23	14 6/12	...	2	...	...	..	...	1	1	...	...	..
BCP	4	1	5	7	48	28'0	9/12	43	21 6/12	1	2	...	...	2	..	1	2	4 6/12	4 6/12	4 6/12
CP	9	10	19	15	60	35'4	1	55	24 2/12	10	3	1	...	5	14	...	...	1	4 6/12	2 2/12
P	1	...	1	...	...	15'0	...	...	13	...	...	..	...	1	...	...	...	...	...	9/12
SL & SL+	8	5	13	6	80	32'0	4	30	11 7/12	6	1	2	...	4	7	1	1	1	4 11/12	3 7/12
BG	1	...	1	...	...	60'0	...	...	40	...	...	...	1	...	1	...	..	...	...	...
BCG	1	...	1	...	...	12'0	...	...	7	..	1	...	...	...	1	...	...	...	...	...
CG	...	2	2	15	25	20'0	4	13	8 9/12	...	...	...	...	2	..	...	...	...	...	...
G	17	8	25	9	40	25'6	2	38	17 6/12	12	2	3	1	7	18	...	...	5/12	4 7/12	3 1/12
	264	155	419	...	...	...	...	...	...	198	95	18	3	105	185	42	49	...	...	2 9/12+

unob = unobserved; uc = uncured; pc = practically cured; c = cured



These various cases have been grouped according to the several lesions of yaws which they presented when first seen in 1920, and the groups are arranged in what we think is approximately their chronological sequence.

As pointed out in our previous publication, a considerable number of cases were seen but once in 1920; most of these came during the last few days of our stay, received a single injection, and there was insufficient time before our departure to observe the results of the treatment; there were 105 such cases among the 419 cases in the 1925 series. There were also included in the 419 cases 38 individuals who, in 1920, gave a history of yaws and received treatment although they presented no active manifestation of the disease at that time. Of the remaining 276 cases, 185 were noted as 'uncured'; 42 as 'practically cured' and 49 as 'cured' at the time of the last observation in 1920. The number of injections which these patients had received prior to the last observation is given in the column just preceding the one headed 'Results in 1920.' It should be noted that a considerable number of the cases in 1920 received an injection at the time of the last observation; this final injection is not recorded in the 'number of injections' column in the above table as it could not have influenced the results as noted in 1920 but it is included in the next table as it may have influenced the results as noted in 1925.

It has already been pointed out that the use of the terms 'cured' and 'practically cured' was not intended to express a final judgment as to the result of therapy in 1920 and even in the case of the 1925 observations the term 'cured' is intended only to express freedom from signs and symptoms of the disease from the time of their disappearance which was at, or very shortly after, the last treatment in 1920, to the date of observation in 1925, i.e., a period of approximately five years.

The promptness with which the primary and secondary lesions of yaws, even such late secondary lesions as clavus, disappear after one or two injections of arsphenamine or its modifications, is familiar to all who have had a sufficient experience with the disease. In our experience the average time is about two weeks. If the lesions in the primary or secondary stage persist a month or longer after treatment and then disappear, we are inclined to regard this as a spontaneous

remission or perhaps a remission influenced to some extent by treatment, but not a cure. In these cases lesions are very apt to recur after a shorter or longer free interval.

We have no data on the length of the free interval which may follow a spontaneous remission in the course of the disease but where any free interval occurred following treatment in 1920 among the cases which we designated as 'uncured' in 1925, we have shown in the above table the minimal, maximal and average free interval for each group. The average free interval for the 195 patients which represents the total uncured group was 2 years 9.6 months, and from the histories obtained we suspect that this is considerably longer than the average free interval following a spontaneous remission.

There were four cases in which cure seemed to have followed treatment in 1920 and a subsequent re-infection occurred. The data on these four cases are presented in the following table.

Serial number	Observations in 1920						Observations in 1925			
	Sex	Age	Diag	Dur. years	Number injects	Result	Cured after	Free interval	First return lesion	Physical exam
642	M	1	MB'C	1	2	Improved	1 week	Years 4 <sup>9</sup> / <sub>12</sub>	M	M
472	M.	9	B'	1	2	Cured	.	4 <sup>5</sup> / <sub>12</sub>	M	M
439	F	7	B	.	2	Much improved	1 week	3 <sup>6</sup> / <sub>12</sub>	M	MB
268	M.	8	C	2	1	Unobs	2 weeks	4 <sup>10</sup> / <sub>12</sub>	M	M

It will be noted that all of these patients were young, that the duration of the disease at the time of the treatment in 1920 had not exceeded two years in any case; that the lesions disappeared promptly after treatment and there ensued a long interval in which they were entirely free from signs and symptoms of yaws and that on examination all presented lesions which strongly suggested the primary lesion and that one patient had secondary granulomata.

In the next table the results of the 1920 observations are compared with those of 1925.

1920														1925													
Diagnosis	No. of cases	Uncured. No. of injections				Cured and p.c. No. of injections				Unobserved	Uncured	Cured and p.c.	Per cent. cured & p.c.	Uncured	Cured	Per cent. cured	Uncured. No. of injections					Cured. No. of injections					
		1	2	3	4	1	2	3	4								1	2	3	4	5	1	2	3	4	5	
M	5	1	1	...	...	2	1	...	...	2	3	...	3	2	...	...	2	1	...	...	...	2	...	...	...		
	5	1	1	...	...	2	1	...	...	2	3	60.0	3	2	40.0	...	2	1	...	...	...	2	...	...	...		
MB'	12	3	...	...	...	4	3	...	...	2	3	7	9	3	...	1	8	...	...	...	2	1	...	...	...		
B'	36	5	3	...	...	13	11	1	...	3	8	25	20	16	...	4	13	3	...	...	1	11	4	...	...		
	48	8	3	...	...	17	14	1	...	5	11	32	29	19	39.1	5	21	3	...	...	3	12	4	...	...		
MB	2	1	...	1	...	...	...	...	...	2	...	...	1	1	...	...	...	1	...	...	1	...	...	...	...		
B	23	6	...	...	...	1	7	1	...	8	6	9	11	12	...	3	6	2	...	...	6	5	1	...	...		
	25	7	...	1	...	1	7	1	...	8	8	9	53.0	12	13	52.0	3	6	2	1	...	6	6	1	...	...	
MB'C	6	2	...	...	...	1	2	...	...	1	2	3	4	2	...	1	3	...	...	...	1	1	...	...	...		
B'C	9	1	...	...	...	1	6	...	...	1	1	7	5	4	...	1	4	...	...	...	3	1	...	...	...		
NBC	1	...	...	...	...	1	...	...	...	1	...	1	1	...	...	1	...	...	...	...	...	...	...	...	...		
BC	47	20	7	...	...	3	7	...	...	10	27	10	22	25	...	7	12	3	...	...	4	17	4	...	...		
C	149	59	20	1	...	6	6	3	1	53	80	16	63	86	...	27	34	2	...	...	30	39	16	1	...		
C+	21	9	2	2	...	1	1	...	...	6	13	2	7	14	...	4	2	1	...	...	2	7	4	1	...		
	233	91	29	3	...	11	23	4	1	71	123	39	24.0	102	131	56.0	40	56	6	...	36	67	26	2	...		
H	30	...	...	...	...	...	...	...	...	...	...	...	9	21	...	9	...	...	...	...	16	4	1	...	...		
H+	8	...	...	...	...	...	...	...	...	...	...	...	5	3	...	4	1	...	...	...	1	1	1	...	...		
	38	...	...	...	...	...	...	...	...	...	...	...	14	24	63.0	13	1	...	...	...	17	5	2	...	...		
MB'CP	1	...	...	...	...	1	...	...	...	...	1	...	1	...	...	...	1	...	...	...	...	...	...	...	...		
BP	2	...	...	...	...	2	...	...	...	...	2	...	2	...	...	...	...	...	...	...	1	1	...	...	...		
BCP	5	...	...	...	...	1	2	...	...	2	3	...	2	3	...	1	1	...	...	...	1	1	...	...	...		
CP	19	10	3	1	...	...	...	...	...	5	14	...	5	14	...	2	2	...	1	...	3	8	3	...	...		
P	1	...	...	...	...	...	...	...	...	1	...	...	1	...	...	1	...	...	...	...	...	...	...	...	...		
	28	10	3	1	...	2	4	...	...	8	14	6	30.0	9	19	68.0	4	4	...	1	...	4	10	5	...	...	
SL & SL+	13	6	1	...	...	2	...	...	...	4	7	2	4	9	...	2	2	...	...	...	2	4	2	1	...		
	13	6	1	...	...	2	...	...	...	4	7	2	22.0	4	9	69.0	2	2	...	...	...	2	4	2	1	...	
BG	1	...	...	...	1	...	...	...	...	1	...	...	1	...	...	...	...	...	...	1	...	...	...	...	...		
BCG	1	...	1	...	...	...	...	...	...	1	...	...	1	...	...	...	...	...	...	...	...	1	...	...	...		
CG	2	...	...	...	...	...	...	...	...	2	...	...	2	...	...	...	...	...	...	...	2	...	...	...	...		
G	25	12	2	3	1	...	...	...	...	7	18	...	21	4	...	5	11	2	3	...	2	1	...	...	1		
	29	12	3	3	2	...	...	...	...	9	20	...	22	7	24.0	5	11	2	3	1	4	1	1	...	1		
	...	135	40	8	2	33	49	8	1	105	185	91	...	195	224	...	72	103	14	5	1	72	107	41	3	1	
	419	185	67%			91	33%			381	...	...	419	...	...	195	46.5%				224	53.5%					

Attention is again called to the fact that the results in 1925 were recorded in many cases after one more injection of neosalvarsan than the patient had received at the time of recording the results in 1920.

Of the present series of 419 cases, 105 were not observed in 1920 after they had received treatment. In addition to these there are included in this table 38 cases who gave a history of yaws in 1920 but who had no active lesions at the time of observation; they were seen and treated during what we considered to be a period of spontaneous remission, therefore the result of treatment could not be observed at that time. Subtracting these two groups there are left 276 cases of which 185 (67 per cent.) were apparently uncured and 91 (33 per cent.) apparently cured or practically cured.

Of the 419 cases observed in 1925, 195 (46.5 per cent.) remained uncured after five years and 224 (53.5 per cent.) were apparently cured; i.e., they had been free from signs and symptoms of yaws for approximately five years.

A slight error may have been introduced in the above analysis by including the cases who gave a history of having had yaws but presented no active lesions at the time of observation in 1920. That this error cannot be a very material one is evident from the fact that there are only 38 cases in this group and the percentage of 'cures' in this group (63 per cent.) is not greatly in excess of the percentage of cures in the entire series (53.5 per cent.).

The number of cases in some of the groups in the above table are admittedly too small to be of much statistical value but the data are given for what they are worth. In considering the stage to which the disease had progressed it seems logical to denote this stage by the most advanced lesion present; for example, if we group the MB' and the B' cases together there are 48 cases which had reached but not passed the stage of the florid secondary eruption. Similarly if we group all the cases which showed clavus as the most advanced lesion irrespective of the fact that in some of these cases earlier lesions of the disease persisted there are 233 cases which had reached but not passed the clavus stage of the disease.

Tabulated in this way and giving the percentage 'cured' (or 'practically cured') in 1920 and 1925, an interesting tendency to a reversal of these percentages according to the stage of the disease is apparent.

1920					1925		
				No. of cases	Cured and practically cured	Cured	No. of cases
					%	%	
M	...	...	...	5	60.0	40.0	5
B'	...	...	...	43	74.4	39.5	48
B	...	...	...	17	53.0	52.0	25
C	...	...	...	162	24.0	56.0	233
P	...	...	...	20	30.0	68.0	28
SL	...	...	...	9	22.0	69.0	13
G	...	...	...	20	...	24.0	29

In our previous report on a much larger series of cases the figures seemed to show just what we anticipated they should show, namely, that the earlier the stage of the disease the more amenable it would be to treatment. Observations made five years later on a smaller series seem to indicate that with the exception of the tertiary stage just the reverse of this is true. A much larger series of cases observed over a longer time will be necessary to settle this point.

In this connection it should be pointed out that in the tertiary stage of the disease 'cure' is exceedingly difficult to judge by clinical observation. Many of these patients have been left in a pitiable state of deformity by the ravages of the disease aided, in many cases, by secondary infections. Eyes may have ulcerated out, the nose may have been eaten away, fingers and toes lost, sinuses reaching to the bones may have persisted for years and even though the patient is sterilised as far as his infection with the *treponema pertenue* is concerned he can never regain the semblance of a sound man.

The 24 per cent. of the 'Gomma' cases recorded as 'cured' were patients whose ulcerations had healed, whose sinuses had ceased to discharge and had closed and who had been free from active signs and symptoms for approximately five years although the evidence of loss of substance and permanent deformities of one sort or another may have persisted. If we had been able to determine the absence of the *treponema* and made this the criterion of cure, the percentage of cures might have been much higher than the figure given for these cases.

In the following table the results as noted in 1920 and 1925 are compared according to the number of injections which the patients had received.

1920								1925									
Uncured				Cured or practically cured				Uncured					Cured				
No. of injections				No. of injections				No. of injections					No. of injections				
1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3	4	5
135	40	8	2	33	49	8	1	72	103	14	5	1	72	107	41	3	1
				19.6%	55%	50%	33.3%						50%	51%	74.5%	37.5%	50%

Of 168 patients who were observed after one injection in 1920, 33 (19.6 per cent.) were recorded as 'cured' or 'practically cured.' Of 89 patients who were observed after two injections in 1920, 49 (55 per cent.) were recorded 'cured' or 'practically cured.' In 1925, 144 patients were observed who had received one injection in 1920, 210 who had received two injections and 55 who had received three injections; the percentages recorded 'cured' in these three groups are 50 per cent., 51 per cent., and 74.5 per cent. respectively. Owing to the smallness of the numbers in the remaining groups, the percentages obtained for them are probably not significant.

It will be noted that as a result of the observations in 1925 the percentages of 'cures' is practically as great in the group which had received one injection as in that which had received two injections (50 per cent. and 51 per cent.), whereas according to the 1920 observations the number of cures was nearly three times as great in the group which had received two injections (55 per cent.) as in the group which had received one injection (19.6 per cent.). This disproportion is probably to be explained by the fact that the patients who received two injections were observed on an average of about ten days longer after their first injection than patients who had received but one injection.

As far as the above figures are of value they suggest that about 50 per cent. of a miscellaneous series of yaws cases made up of patients in the various stages of the disease, may be cured by a single injection

of neosalvarsan ; that the percentage of cures is not much increased by two injections but is increased considerably by three injections. The final judgment on this point will have to be determined by a larger series of cases than that here reported.

The Wassermann test was done in too few cases to be of any special significance. The data are given, however, as they may be of some value in connection with the reports of other authors.

During the summer of 1920 the Wassermann test was performed on the blood of 91 patients. A strongly positive reaction was obtained in 78 cases (85.7 per cent.), moderately positive in 4 cases, weakly positive in 1 case and negative in 8 cases. The reactions according to the stage of the disease are shown in the following table.

Diagnosis	Strongly Positive	Moderately positive	Weakly positive	Negative
M	2	...	...	...
MB'	1	...	...	...
B'	7	1	...	...
B	4	...	...	2
B'C	3	...	...	...
BC	5	...	...	...
C	11	1	...	...
C +	6	...	...	1
H	11	...	...	1
H	4	2	1	1
CP	6	...	...	...
SL	3	...	...	1
BG	...	...	...	1
CG	1	...	...	...
SLG	1	...	...	...
G	13	...	...	1
Total	78	4	1	8

The antigen used was cholesterinized alcoholic extract of human heart muscle, obtained from the Wassermann Laboratory of the Massachusetts State Board of Health. Fresh guinea-pig serum was used as complement, the hemolytic system was immune rabbit serum versus sheep's corpuscles.

In 1925 the Wassermann test was performed on the blood of 73 patients. Blood for this test was taken under sterile precautions, allowed to clot and the serum taken up in sterile glass ampules, hermetically sealed and without refrigeration brought back to the United States, where the tests were performed

in the Wassermann Laboratory of the Massachusetts State Board of Health through the courtesy of Dr. W. A. Hinton, the Assistant Director. The same technique was employed as in 1920 except that the tests were made on serum four to eight weeks old.

The detailed results of the Wassermann tests, together with certain other data, are given in the following table:

1920						1925		1920					1925		
Serial No.	Diagnosis	Duration	Was.	No. of injections	Result	Was.	Result	Serial No.	Diagnosis	Duration	Was.	No. of injections	Result	Was.	Result
866	M	8/12	...	2	i	+	unc	143	C	4	+	2	u	+	unc
306	M	8/12	+	3	mi	-	unc	152	C	1	+	2	mi	+	unc
87	MB'	10/12	...	2	pc	+	unc	162	C	3	...	2	mi	-	unc
113	MB'	4/12	...	2	c	+	c	183	C	5	...	1	unob	+	unc
505	B'	1	...	2	mi	+	unc	198	C	2	...	2	mi	+	unc
908	MB'	11/12	...	2	mi	+	unc	239	C	3	...	1	unob	+	c
26	B'	8/12	...	2	c	+	unc	268	C	...	...	1	unob	+	c
38	B'	8/12	...	2	pc	+	unc	271	C	5	...	1	unob	-	unc
124	B'	8/12	...	2	c	+	unc	313	C	10	+	3	c	-	c
343	B'	2	...	2	pc	-	unc	445	C	25	...	1	unob	+	unc
442	B'	1	...	1	unob	+	unc	482	C	22	...	2	pc	+	unc
906	B'	11/12	...	2	mi	+	unc	520	C	6	...	2	i	+	unc
36	B	8/12	...	2	c	+	unc	538	C	20	...	2	i	-	c
232	B	4	+	0	unob	+	unc	546	C	6	...	1	unob	-	unc
305	B	1	+	2	c	-	c	557	C	9	...	2	i	+	unc
314	B	10	...	2	c	+	unc	778	C	6	...	2	i	+	unc
1136	B	2	...	1	unob	+	unc	907	C	4	...	2	mi	+	unc
660	MB'C	8/12	...	1	unob	+	unc	999	C	11	...	1	unob	-	unc
39	B'C	7/12	...	3	pc	-	c	1138	C	3	...	1	unob	-	c
62	B'C	8/12	+	2	c	-	unc	25	C+	16	...	3	mi	+	unc
58	BC	10/12	+	2	c	-	c	23	H	4	...	1	...	+	unc
167	BC	9	...	3	pc	-	unc	140	H	3	+	2	...	-	c
238	BC	5	...	1	unob	+	unc	233	H	4	+	1	...	+	unc
345	BC	6	...	2	mi	+	unc	451	H	1	...	1	...	-	c
353	BC	5	...	2	i	+	c	948	H	10/12	...	1	...	-	c
405	BC	5	...	2	mi	-	c	552	H+	2	...	3	...	-	c
528	BC	15	...	1	unob	-	unc	905	H+	3	...	1	...	+	unc
657	BC	7	...	1	unob	+	c	68	CP	3	...	2	u	-	unc
670	BC	2	...	2	mi	+	unc	368	CP	22	...	4	i	-	unc
786	BC	2	...	2	mi	-	c	213	SL	20	+	4	pc	+	c
967	BC	5	...	1	unob	+	unc	70	G	2	...	2	i	+	unc
20	C	3	...	1	unob	-	unc	237	G	16	+	4	mi	-	unc
35	C	25	...	3	i	-	unc	596	G	18	...	2	u	+	unc
61	C	4	+	3	mi	+	unc	874	G	24	...	2	u	-	unc
79	C	2	...	1	unob	-	unc	956	G	10	...	2	u	-	c
137	C	3	+	2	i	-	c	1074	G	9	...	1	unob	-	unc
142	C	8	...	2	i	-	unc								

Results: — c = cured; pc = practically cured; mi = much improved, i = improved,  
u = unimproved; unob = unobserved; unc = uncured.



The above table shows that of the seventy-three cases on which a Wassermann test was done in 1925 there were only fourteen on which the test was done in 1920. Of these, four gave a positive test both times and all of these were recorded as "uncured" from the history and physical examination made in 1925, before the result of the final Wassermann was known. Two patients gave a positive test in 1920 and a doubtful test in 1925; of these one was recorded as 'cured' and the other as 'uncured.' Eight patients gave a positive test in 1920 and a negative test in 1925. Five of these were recorded as 'cured' and three as 'uncured' as a result of history and physical examination.

If the entire series of seventy-three patients on whom the Wassermann test was performed in 1925 is examined with reference to the results as judged by history and physical examination, the following resumé is obtained from the above table: of twenty-nine cases who gave a positive Wassermann, twenty-five were recorded as 'uncured' and four cases as cured (14 per cent.); of eleven cases who gave a doubtful reaction, nine were recorded 'uncured' and two as 'cured' (18 per cent.); of thirty-three cases who gave a negative reaction, nineteen were recorded as 'uncured' and fourteen as 'cured' (42 per cent.).

That a considerable percentage of negative reactions (58 per cent.) was found in uncured cases five years after insufficient treatment is not surprising.

The fact that four of the twenty-nine cases who gave a positive reaction in 1925, were, as a result of history and physical examination, recorded 'cured' may indicate that our percentage of 'cures' is too high.

### RESUMÉ

During the summer of 1920 Drs. Sellards, Bigelow and the author had the opportunity of studying and treating 1,046 cases of yaws occurring in the Dominican Republic.

Neosalvarsan, usually given intravenously, was the therapeutic agent employed. The usual dose for an adult was 0.6 grams. In children the dose was roughly proportioned to age and body weight.

In 570 cases of this series the result of treatment was noted at

times ranging from about one to six weeks after the first injection. Irrespective of the number of injections given there were 176 cases (30.88 per cent.) recorded 'cured' or 'practically cured.' Of 362 cases observed after a single injection, 72 (19.8 per cent.) were recorded as 'cured' or 'practically cured' and of 169 cases observed after two injections, 87 (51.5 per cent.) were recorded as 'cured' or 'practically cured.'

The results of the 1920 observations seemed to indicate that the earlier the stage of the disease the more amenable was it to neosalvarsan therapy.

Of the 1,046 cases studied in 1920, the author had the opportunity of making further observations on 419 during the summer of 1925.

Of these 419 cases 105 were not observed in 1920 after they had received treatment and there are included also 38 cases who gave a history of yaws but who presented no active manifestation of the disease at the time they were observed in 1920. Subtracting these two groups there are left 276 cases, of which 185 (67 per cent.) were apparently uncured and 91 (33 per cent.) recorded as 'cured' or 'practically cured' at the last observation in 1920.

In 1925 observations were made on the 105 cases who escaped observation in 1920 and also on 38 cases who gave only a history of yaws in 1920. Of the entire series of 419 cases, 195 (46.5 per cent.) remained uncured five years after they had received treatment and 224 (53.5 per cent.) had been free from signs or symptoms of yaws for approximately five years.

In analysing the results of the 1925 observations according to the stage of the disease a tendency to a curious reversal of the percentage of 'cures' is noted, namely: that with the exception of the 'goma' cases, the later stages seem more amenable to neosalvarsan therapy than do the earlier stages. Further observations are necessary to settle this point.

The results as observed five years after treatment suggest that about 50 per cent. of a miscellaneous series of yaws cases made up of patients in the various stages of the disease may be cured by a single injection of neosalvarsan, and that the percentage of cures is not much increased by two injections but is considerably increased by three injections. Before these results can be accepted as establishing a rule a larger series of cases must be studied.

The Wassermann test performed on 91 patients in 1920 gave the following results: strongly positive in 78 cases (85.7 per cent.), moderately positive in 4 cases, weakly positive in 1 case and negative in 8 cases. The 8 negative Wassermanns were given by patients in the late secondary or tertiary stage of the disease.

In 1925 the Wassermann test was done on the blood of 73 patients of whom one did not receive an injection of neo-salvarsan in 1920, 23 had received one injection, 38 had received two injections, 8 had received three injections, and 3 had received four injections. Of the 73 patients tested, 29 gave a positive reaction, 4 of whom had been free from symptoms and signs of yaws for approximately five years, 11 gave a doubtful reaction, 2 of whom had been free from symptoms and signs of yaws for approximately five years; 33 gave a negative reaction, 14 of whom had been free from symptoms and signs for a like period.

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# A STUDY OF CUTANEOUS LEISHMANIASIS IN PALESTINE

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## PLATES XXX-XXXII

Cases of cutaneous Leishmaniasis in Palestine are either imported from neighbouring countries or they are acquired in Palestine itself. Of the hundred cases\* which are dealt with in the present paper, twenty-eight were acquired locally, the remainder were imported from :—

Persia	...	...	...	31 cases
Mesopotamia	...	...	...	36 „
Syria	...	...	...	1 case
Egypt	...	...	...	1 „
Afghanistan	...	...	...	1 „
Samarkand	...	...	...	1 „
Transjordan	...	...	...	1 „
—				72 cases
				—

There is no absolute certainty as to the source of the infection in all the patients from Persia for all these pass through such endemic centres as Baghdad or Aleppo on their way to Palestine.

The cases acquired in Palestine come from various localities which, with the exception of Jericho, have only recently been discovered to be foci of disease.

\* Our material reached 127 cases, but full details were obtained in only 100 cases.

Kligler (1923) found three cases of the disease in Kantara (30 kilometres south of the boundary of Palestine).

Dostrowsky (1925), during 1923-1924, found ten cases in Artuf, a village 22 kilometres from Jerusalem, with a population of 114. During 1925 another case (referred to below as Case III) was found in this village.

The author (1925) expressed his opinion that cutaneous Leishmaniasis in Palestine was not confined to limited and circumscribed areas and that careful clinical and microscopical observations would disclose new foci throughout the whole country. The following clinical histories support this view.

CASE I. Card No. 1332, female, 19 years old from Mozza, a Jewish village seven kilometres from Jerusalem (2,115-2,570 feet above sea level).

The case was first seen 11.5.24. On the left forearm an ulcer 0.5 cm. in diameter. The subcutaneous tissue was indurated, the ulcer was surrounded by a reddish infiltrated area. The patient had never been to Jericho but had made several trips to Jaffa and Jerusalem. She had noticed the ulcer eleven weeks before reporting to the clinic. No other signs or symptoms were noted. Leishman-Donovan bodies were found in a smear of the ulcer.

CASE II. Plate XXX, fig. 1. Card No. 1966. Male, 10 years old, from Beth-Djalla, 8 kilometres from Jerusalem, 1,690 feet above sea level. A village near Bethlehem. First seen 30.7.24.

An almost round ulcer 2 cm. in diameter covered with sero-haemorrhagic crust; on removal of the crust the margins of the ulcer were found to be prominent and not undermined; the floor of the shallow ulcer was covered with papillary granulations. The margin of the ulcer was surrounded by a reddish infiltrated area, 0.5 cm. wide, elevated above the surrounding normal skin. The lesion was indurated but not attached to the subcutaneous tissue. The patient gave a history of three months. He had never been in Jericho, but had visited Jerusalem several times. Leishman-Donovan bodies were found in a smear from the ulcer.

CASE III. Card No. 11776. Male, 41 years old, from Artuf, 22 kilometres from Jerusalem, 1,090 feet above the sea level.

The case was first seen 26.3.25. On the left ankle an ulcer the size of a shilling piece covered with a seropurulent crust. The area round the ulcer was swollen. The duration of the ulcer was three and a half months. Leishman-Donovan bodies were found in a smear.

CASE IV. Plate XXX, fig. 2. Card No. 337. Male, 15 years old, from Beth-Djalla.

First seen 28.10.25. One irregular ulcer on the face and one on the left forearm. Both were typical oriental sores. Duration three months. The patient had never visited Jericho. Numerous Leishman-Donovan bodies were found in smears from both ulcers.

**CASE V.** Plate XXXI, fig. 1. Card No. 9561. Female, eight and a half years old.

First seen 31.1.26. An elevation 1 cm. in diameter covered with scales a serous crust was found on the nose. The lesion was indurated, indolent and surrounded by a brownish-violet area. A similar lesion was found on the leg dorsally. During the last two years has never left Jerusalem. The patient had never visited Jericho. Numerous Leishman-Donovan bodies were found in smears of both lesions. The lesions first appeared in September, 1925. The family at the time lived in Beth-Hakerem, a new suburb of Jerusalem three kilometres west of the city.

**CASE VI.** Card No. 1427. Male, 14 years old, brother of Case V.

First seen on April 25, 1925. On the right side of the neck, an oval erythematous elevation 0.8 cm. long was found. The centre of the lesion was hard and indolent. Leishman-Donovan bodies were found in the lesion. The lesion, according to statement of the patient, appeared in February, 1926, six months after the patient's family left Beth-Hakerem and settled in the city. The patient had never been to Jericho.

**CASE VII.** Card No. 1374. Female, 6 years old, sister of Cases V and VI.

Seen on 25.3.26. A hard indolent bluish-red button-like nodule about the size of a bean and covered with scales was seen on the right cheek. On the left cheek two small similar lesions. The nodules were first noted in January, 1926. The patient had never visited Jericho. Leishman-Donovan bodies found in a smear of the lesion.

**CASE VIII.** Card No. 2358. Female, 20 years old.

Seen on 1.7.26. On the right forearm under the olecranon process a hard bluish-red nodule covered with smooth skin (no scales). The lesion was indolent and showed induration down to the subcutaneous tissue. History four months. Another indurated lesion 2 cm. in diameter ulcerated in the centre and covered with a sero-haemorrhagic crust and surrounded by a bluish-red margin was found immediately below the first lesion. Both lesions were painless. History 9 months. Tissue was removed from the first lesion and on section Leishman-Donovan bodies were found.

Patient, a native of Roumania, arrived in Palestine, May, 1925, and lived in Tel-Aviv two weeks and then two months in Beth-Hakerem in the same house which case V occupied at the time. She then returned to Tel-Aviv for three months and subsequently lived in Jerusalem. The patient had never visited Jericho.

**CASE IX.** Dr. A. Beham, Director of the Pasteur Institute, Jerusalem, kindly reported to the author a case of cutaneous Leishmaniasis from Sechem. Patient, female, 22 years old, showed two lesions, one a small ulcer and the other a nodule on the left side of the forehead above the eye. Three other lesions were found on the left leg near the external malleolus. Leishman-Donovan bodies were found in all the lesions. The patient had never left Sechem during the previous eighteen years, and had never visited Jericho.

It is quite probable that owing to non-differentiation between oriental sore and other lesions of the skin the number of cases in Palestine is really greater than is at present suspected.

The number of parasites in different cases was very variable, in some cases a smear was found swarming with Leishman-Donovan bodies and in others these bodies were found after a search of several hours. There were cases diagnosed clinically and microscopically as *Leishmania cutis* and labelled by me as cases of Jericho boils only because they had passed through Jericho on a motor trip taken two years before the appearance of the lesions. It seems doubtful in the light of our recent findings whether these cases were really acquired in Jericho.

On the other hand there were cases seen before 1924 which clinically seemed typical cases of oriental sore though they had never visited Jericho, and Leishman-Donovan bodies were not found. Only a single examination was made at that time because the author was under the impression that all cases of cutaneous Leishmaniasis in Palestine were acquired in Jericho.

The facts stated above prove that cutaneous Leishmaniasis is present in Palestine throughout a large area of the country and it is interesting to note that this area shows very large variations in geographical and meteorological conditions. Jericho (820 feet below sea level), with a tropical climate, Jerusalem (2,593 feet above sea level), with a temperate climate, Kantara (150 feet above sea level), localities which differ from each other meteorologically, are all foci of the disease (see map). The following is a brief clinical description of the disease as observed in Jerusalem.

The number of sores in a single individual varied from one to one hundred and fifty eight. The lesions consisted of elevations of the skin varying in size from a small papule to sores  $13\frac{1}{2}$  cm. in diameter (Plate XXXI, fig. 2). The lesions observed in the outpatient department were of various stages of development and duration. The more recent cases (when free from secondary infection) showed reddish or bluish-red non-ulcerating, flat, indurated and indolent tubercles, the more advanced cases showed indurated ulcers (the induration included the subcutaneous tissue) covered with serous, sero-haemorrhagic (where secondary infection was present, with sero-purulent) crusts and surrounded by a brownish or bluish-red halo.

Cases of *Leishmania verrucosa* and *Leishmania framboesiformis* were noted to occur.

Only three cases showed infection of a mucous surface, all of them in the mucosa of the lower lip. Lesions free from secondary infection did not give rise to symptoms.

Cases from Jericho, Artuf, Baghdad, Aleppo, Egypt and Persia all showed a similar clinical picture well known from the literature on the subject. Differences in the clinical manifestations of various cases depend either on variations in the virulence of *Leishmania tropica* or chiefly on the peculiarities of the skin of infected individuals, and are in no way related to the locality where the infection was acquired. This view is also supported by the recent work of Kligler (1926), who showed that the parasites from oriental sores acquired in various localities (Palestine and Baghdad) were biologically identical.

Dr. Adler informed me that in one case experimentally induced by injection of *Herpetomonas* from *Phlebotomus papatasi* a subcutaneous nodule was produced (the lesion was examined by the author) and that further inoculation from this lesion into another human case produced a papule. It appears therefore that variations in individual human beings are a more important factor than variations in *Leishmania tropica*.

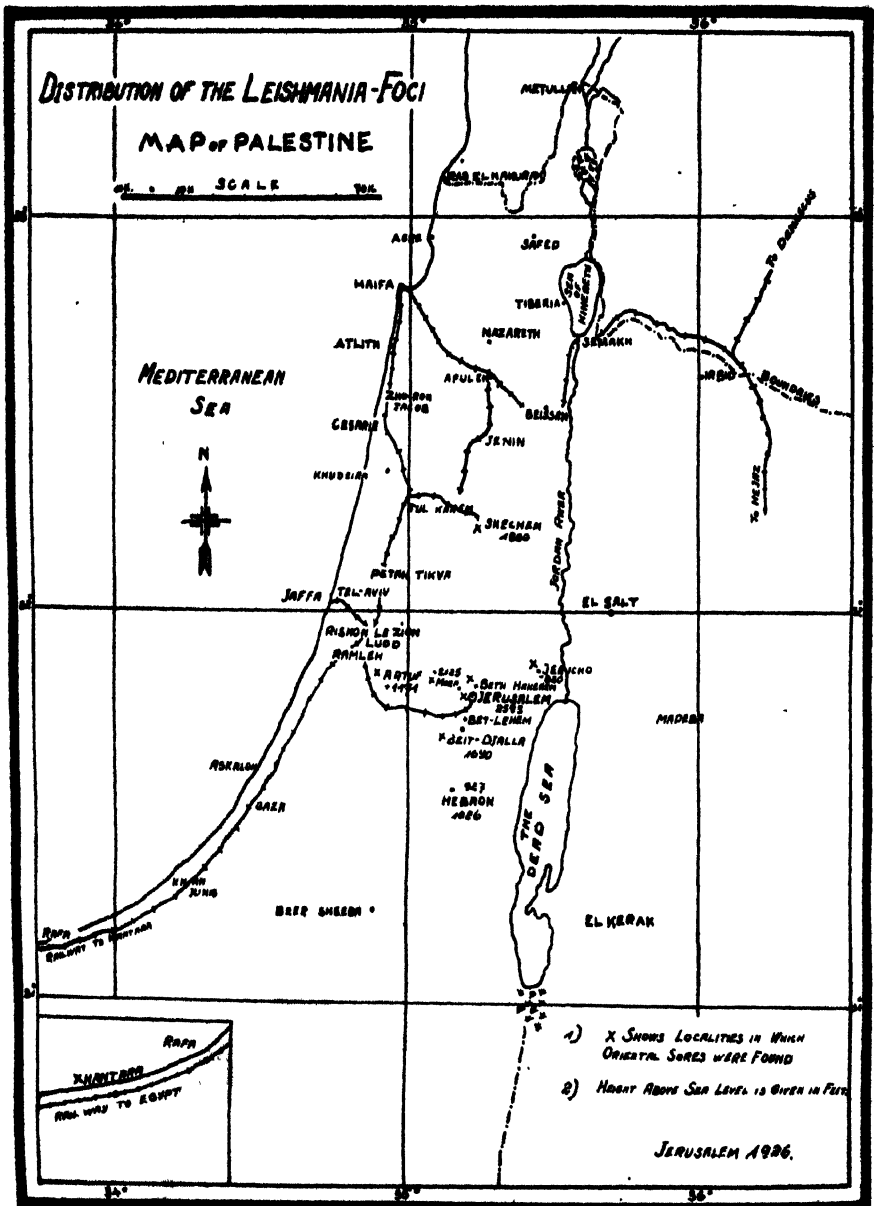
In several cases with numerous lesions in the upper limbs the supratrochlear and the axillary glands were enlarged and hard, but painless. In one case (No. 1124) (Wassermann reaction negative) where the supratrochlear gland was excised, no Leishman-Donovan bodies were found.

In Case 27 a Wassermann reaction was made by Dr. Yunowitz, in the laboratory of the Rothschild Hospital (Dr. A. Felix). The results are shown in the following table:—

TABLE I.

Results	...	...	...	...	...	+++	++	+	↓	?	—
No. of case	...	...	...	...	...	2	3	4	9	1	8





None of the above cases showed symptoms nor did they give a clinical history of lues. Case No. 522, which showed a strong W.R., was refractory (in respect to the Leishmaniasis) to neo-salvarsan, but responded readily to tartar emetic. The degree of the Wassermann reaction showed no relationship to the number of oriental sores. Thus Case No. 522, W.R. + + +, showed 158 boils, while Case No. 1769, W. R. + + +, showed only one lesion. Case No. 1127 (Plate XXXII) which had an enlarged supratrochlear gland showed unexpectedly a negative Wassermann before and after treatment.

The results of the Wassermann reaction, although made on a small number of cases, show that in cutaneous Leishmaniasis a large percentage of cases show changes which give rise to a positive reaction in the serum. The relationship of oriental sores to the Wassermann reaction requires further investigations, for should a definite positive relationship be established the interpretation of the W.R. in endemic centres of oriental sore will be complicated.

We were unfortunately not able to determine the ultimate result of treatment (for oriental sore) on the W.R. for the cases did not return to the clinic after completion of treatment.

Dr. S. Adler, of the Microbiological Institute, Jerusalem, informs me that he has found Napier's formaldehyde reaction negative in sixteen cases of oriental sore, thirteen natural infections and three artificial infections. This result, which is in marked contrast to the findings in Kala-Azar, adds to the already ample evidence of the biological difference of *Leishmania tropica* and *Leishmania donovani*.

The appearance of new foci of oriental sore is of epidemiological rather than of clinical interest. Of particular interest is the localisation of the lesion which in many exanthemata provides useful aetiological information. Many authors have rightly attached great importance to the distribution of the lesions in cutaneous Leishmaniasis. The accompanying fig. 1 and Table II show the distribution of the lesion in the cases under the author's notice.

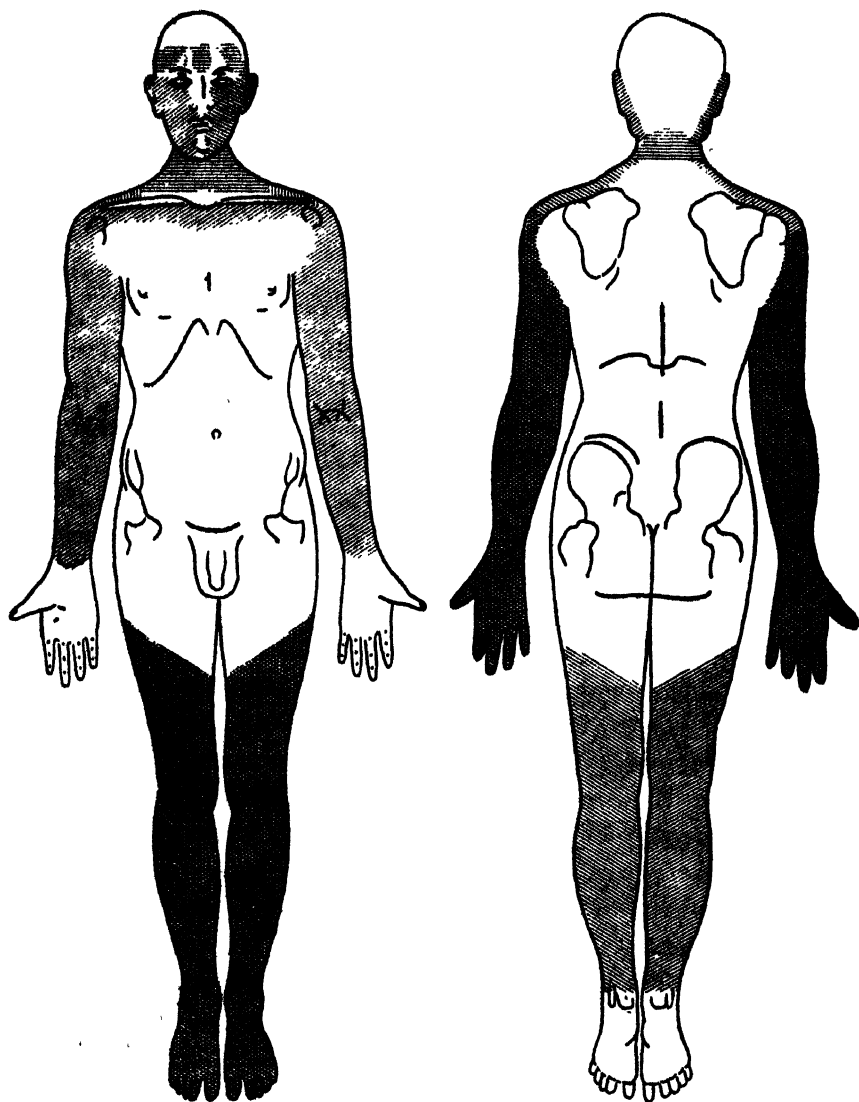


FIG. 1. Anatomical Localisation of Sores graphically represented; showing the dorsal localisation on the extremities.

TABLE II.

Distribution of Leishmania Sores in different parts of the body in 100 cases.

	Palestine		Persia		Mesopotamia		Syria		Samarkand		Trans-jordania		Egypt		Afghanistan		Total in all of the countries		Total in both sexes	Total %
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.		
No. of cases...	14	14	18	13	20	16	1	...	1	...	1	...	1	...	1	...	57	43	100	
Localisation :																				
Scalp .....	24	12	19	15	26	44	1	...	1	...	1	...	1	...	1	...	74	71	145	23.3
Neck ...	4	...	1	1	2	4	...	...	1	...	...	...	...	...	...	...	8	5	13	2.1
Trunk ...	...	...	...	2	...	2	...	...	...	...	...	...	...	...	...	...	...	4	4	0.6
Arm ...	...	14	...	...	1	21	...	...	...	...	...	...	...	...	...	...	1	35	36	5.8
Forearm ...	46	28	23	20	35	70	...	...	...	...	...	...	...	...	...	...	104	118	222	35.6
Hand ...	8	6	5	...	11	27	...	...	...	...	...	...	...	...	...	...	24	33	57	9.1
Thigh ...	...	2	1	...	2	17	...	...	...	...	...	...	...	...	...	...	1	19	22	3.5
Leg ...	...	14	8	2	4	51	1	...	...	...	...	...	...	...	...	...	13	67	80	12.8
Foot ...	1	2	4	6	6	24	1	...	...	...	...	...	...	...	...	...	12	32	44	7.1
Total No. of sores ...	83	78	61	46	87	260	3	...	2	...	1	...	1	...	1	...	239	384	623	100.0

TABLE III.

Distribution of sores on extremities in 36 cases.

Localisation						Palestine, 16 cases		Exogenous, 20 cases		Total	
						Dorsal	Volar	Dorsal	Volar	Dorsal	Volar
Arm	...	...	...	...	...	2	...	8	2	10	2
Forearm	...	...	...	...	...	10	...	58	14	68	14
Hand	...	...	...	...	...	4	...	15	...	19	...
Thigh	...	...	...	...	...	2	...	10	...	12	...
Leg	...	...	...	...	...	2	...	34	3	36	3
Foot	...	...	...	...	...	1	...	22	...	23	...
Total	...	...	...	...	...	21	...	147	19	168	19

The table proves that *the lesions are most common on the extremities and face and are very rare on the body* (Plate XXXII). Only 0.6 per cent. were found on the upper and anterior part of the thorax. The distribution of the lesions is as follows : forearm (35.6 per cent.), face (23.3 per cent.), leg (12.8 per cent.), hand (9.1 per cent.), foot (7.1 per cent.), upper arm (5.8 per cent.), thigh (3.5 per cent.), neck (2.1 per cent.).

*On the extremities the lesions were noted to be most prevalent on the dorsal aspects.* Out of 36 cases in which the distribution on the extremities was particularly studied, 168 out of a total of 187 lesions on the extremities (i.e., 89 per cent.) were dorsal. *Volae manus and plantae pedis were never affected.* (Table No. III.)

Several authors have considered the presence of single or multiple lesions as characteristic of various endemic foci. Our material collected in sufficient numbers from various foci disproves this view, e.g., out of 37 cases from Persia 13 had single lesions, out of 35 cases from Mesopotamia 11 had single lesions, out of 9 cases from Jericho 3 had single lesions, out of 17 cases from Palestine (apart from Jericho) 5 had single lesions. *There are no foci characterised by single or multiple lesions.*

The actual date of the appearance of the lesion is in many cases difficult to determine, for the appearance of small papules often escapes the attention of intelligent patients in good circumstances and is entirely overlooked by labourers who report only when, owing to its large size or to secondary infection, the lesion interferes with their work (the bulk of our patients were labourers). The date of reporting to the outpatient department is of no aetiological importance, being entirely dependent on subjective factors. We cannot therefore make use of the seasonal factor in determining the aetiology of the disease.

In 74 cases, however, we attempted on the basis of information, which at best leaves room for error, to determine the date of the appearance of the lesion. As will be seen from Table IV, there is no great difference in the appearance of the lesion according to the months of the year.

The conclusion may be drawn (with no great certainty) that 65 per cent. of the lesions appear between September and April, i.e., immediately before and during the rainy season. In the cases

acquired in Palestine where the information could be controlled the date of noting the lesions by the patient also gave no certain seasonal information of aetiological value.

TABLE IV.  
Monthly distribution of occurrence of Leishmania Sores.

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total
1921 ...	1	...	...	...	...	...	2 (1)*	...	...	...	...	...	3 (1)
1922 ...	...	...	...	...	...	...	1	...	...	1	1	...	3
1923 ...	3	...	1	2 (1)	3 (1)	3	...	1	2 (1)	(1)	1	1	18 (4)
1924 ...	6 (5)	2 (2)	...	1 (1)	2 (1)	1	4	2	2	3 (3)	...	1	24 (12)
1925 ...	...	1	2 (1)	...	1	...	...	3	4 (2)	4	5	1	21 (3)
1926 ...	4 (2)	1 (1)	...	...	...	...	...	...	...	...	...	...	5 (3)
Total ...	14 (7)	4 (3)	3 (1)	3 (2)	6 (2)	4	7 (1)	6	8 (3)	9 (4)	7	3	74 (23)

\* The figures in brackets represent the Palestinian cases.

TABLE V. Age incidence.

Age	Jericho	New Palestine foci	Persia	Mesopotamia	Afghanistan	Samarkand	Trans-jordania	Egypt	Syria	Total
0-5 ...	...	2	5	...	1	...	...	...	...	8
5-10 ...	...	4	6	4	...	1	...	...	1	16
10-15 ...	...	4	7	12	...	...	...	...	...	23
15-20 ...	3	3	...	6	...	...	...	1	...	13
20-30 ...	5	2	5	7	...	...	1	...	...	20
30-40 ...	...	1	3	4	...	...	...	...	...	8
40-50 ...	...	2	3	3	...	...	...	...	...	8
50-60 ...	1	1	1	...	...	...	...	...	...	3
60-70 ...	...	...	1	...	...	...	...	...	...	1
Total ...	9	19	31	36	1	1	1	1	1	100

Table V shows that in endogenous and exogenous cases all ages are affected with cutaneous Leishmaniasis, but the table does not give the real age distribution of the disease for most of the cases are immigrants and immigration is selective. The probability is that the infection rate among children is greater than the table shows. The numbers from Jericho are small for the population of Jericho does not as a rule come to the Jerusalem clinics. The nine cases from Jericho were not local but acquired the disease during a short stay in that town.

An interesting phenomenon noted was the appearance of the disease in families, i.e., 24 of the cases were in 7 families. In our opinion these figures are probably an underestimate for it was not found possible to examine all the members of patients' families. The cases were naturally selective, for while a girl with an oriental sore on her face hastens to the out-patient department, a labourer with a sore on his forearm, knowing the disease not to be serious, does not report at all. The endemic focus in Artuf (10 cases) was discovered only because a lady had an oriental sore on her face.

There are two possible explanations of the spread of cutaneous Leishmaniasis in Palestine.

1. Cases from Jericho or from other well-known foci of the disease in the Near East infect new cases while passing through the country.
2. The same favourable conditions for the spread of the disease which are present in Jericho also prevail to a lesser extent in other places where the disease occasionally appears.

The first explanation presumes that cutaneous Leishmaniasis spreads by direct contact but in the four cases recorded above the author could trace no history of contact with cases of the disease nor did any of them pass through previously recognised endemic foci of the disease.

The cases imported into Palestine are mostly poor Jewish immigrants from Persia (where the disease is endemic) who pass through Baghdad and Aleppo (also endemic foci) on their way to Palestine. These immigrants on arrival in Jerusalem usually confine themselves to three quarters of the city (Bukharia, Nachlat Zion, Shimon Hazadik) but they come in contact with the rest of the population during their work.

The author found a servant girl from Persia infected with

Leishmaniasis employed in Beth-Hakerem, a new suburb of Jerusalem, where, as previously stated, new cases of the disease were found. From this it may be thought that the new cases of Leishmaniasis in Jerusalem were acquired through direct or indirect contact.

In Cases II and IV (Arab children from Beth-Djalla), 8 kilometres from Jerusalem, and in Case IX in Artuf (22 kilometres from Jerusalem) contact with infected immigrants can be excluded with certainty and in the other cases contact was extremely unlikely.

Johnstone (1925) thought that the presence during 1917-1918 of a number of cases of cutaneous Leishmaniasis in a military hospital in the neighbourhood of Artuf was responsible for the outbreak of the disease in the latter village. This view is not justified, for it was not till 1923 that the first cases occurred in Artuf, i.e., an incubation period longer than any hitherto recorded. Since 1921, a physician was present in Artuf and it is unlikely that in a small population of one hundred and fourteen, chronic ulcers would have passed unnoticed.

Against the contact theory there is the evidence of the well-known fact, that cutaneous Leishmaniasis cannot be inoculated experimentally through unbroken skin.

It is interesting that new cases have not so far been seen in the districts where the immigrants from Persia settled, in spite of the fact that these districts are very poor and overcrowded. It is also interesting to note that the patients and their relatives often state that the sores are not infectious. It appears, therefore, that the presence of a large number of cases in one district is of itself insufficient to cause an epidemic in that district.

An infection through direct contact, even in families, although theoretically possible, can only be in the nature of a rare accident. The occurrence of the disease in families points to a common aetiological factor.

The aetiology of Case IX is of particular interest. Inquiries proved that this case (a schoolmistress) inhabited a room in the same house in Beth Hakerem as Cases V, VI, VII and VIII for three months. Case V first noted the sore on her nose about a month after leaving Beth Hakerem and Case IX, who had also left Beth Hakerem, noted a sore on her forearm at approximately the same



time. No persons from Jericho or other endemic centres inhabited the same house and it is difficult to find the common first cause of these cases.

The above facts speak against the theory of infection by direct contact.

### THE THEORY OF TRANSMISSION BY BITING INSECTS

The facts in favour of this theory have been sufficiently discussed in the literature on the subject. I would briefly emphasize several facts observed in my own clinic which support this view.

1. The localisation of sores on points which are exposed to the bites of insects by night.

2. In cases of oriental sore suffering also from *Pediculus corporis*, and in which there was an obvious opportunity for the spread of the disease by scratching, the sores still showed the characteristic localisation. (This proves that lice are not carriers.)

3. In case of oriental sores on the extremities the dorsal surface was usually attacked.

4. The distal parts of the extremities were usually attacked, the proximal parts (upper arm and thigh) rarely.

Transmission by insects is proved by the experiments of the Sergeants, Ed. and Et., Parrot, L., Begnet, U., and Donatien (1921), de Baurepaix, Aragao (1922), and those of Adler and Theodor (1925). The former observers transmitted cutaneous Leishmaniasis to man by inoculation from an emulsion of sandflies which they considered to be *Phlebotomus papatasi* (the emulsion, however, on examination, was not found to contain *Herpetomonas*) and the latter observers inoculated *Herpetomonas* from sandflies into man and produced in one case a typical oriental sore, and in two cases cutaneous Leishmaniasis which did not appear clinically typical to the author. This experiment should be controlled in other endemic centres in order to corroborate the *Phlebotomus* theory. So far *Phlebotomus papatasi* only has been used for the experimental transmission of oriental sore and it still remains to be determined whether other species of the genus *Phlebotomus* and, indeed, biting insects of other genera are capable of transmitting the disease.

The transmission experiments so far performed are subject to the criticism that the lesions are a Herpetemoniasis of the skin caused by an artificially introduced Herpetemonas which is not biologically identical with *Leishmania tropica*. The relationship between species of Herpetemonas in general and *Leishmania tropica* remains to be determined by biological reactions such as agglutination (Kligler, 1925, 1926, Noguchi, 1925) if possible by complement deviation. (Dr. Adler informs me that the three experimental strains were found to be biologically identical with *Leishmania tropica*.)

Again it still remains to be determined whether *Leishmania tropica* undergoes a biological cycle in sandflies. The work of Adler and Theodor tends to show that there is such a cycle in *Phlebotomus papatasi* but their evidence is not complete and so far applies to one species of sandfly.

The appearance of new cases in hitherto non-endemic centres of the disease can be explained on the *Phlebotomus* theory. In a constant endemic centre of oriental sore the percentage of infected sandflies is relatively high and the probability of human infections is correspondingly high. Thus Wenyon (1912) found 6 per cent. of sandflies infected in Aleppo. In Jericho, a smaller endemic centre, Adler and Theodor found an infection rate with Herpetemonas of one per thousand during 1925 (according to a personal communication of Dr. Adler). In localities where the percentage of infected sandflies is small, cases of oriental sore will be few or incidental as our observations in Palestine show. It is probable that the number of lesions per person is also ultimately dependent on the percentage of infected sandflies: thus in places where cases are few such as Kantara, Mozza, Jerusalem, single sores only were found, while in heavily infected centres multiple lesions are common.

There is yet another factor in the epidemiology of oriental sore which must be borne in mind. The infestation of various places in Palestine with sandflies is not constant. There are great variations due to undetermined causes. In Artuf, for instance, they were numerous. The inhabitants of Mozza stated that 'the small greyish insects which bite and do not make a noise' are not noticed some years and in other years they are a pest. This statement, although made by villagers, cannot be disregarded.

The possibility of sandflies being carried from place to place by transport (railway wagons, etc.) must be considered, although there is no evidence as yet on this subject.

Finally the arrival of cases from an endemic focus to a new locality may give rise to an infection in the local sandflies and thus new cases may be infected.

We have attempted to explain the paucity of locally acquired cases of oriental sore in Palestine on epidemiological grounds, i.e., on a small infection rate with *Herpetomonas* among sandflies.

### SUMMARY AND CONCLUSIONS

1. Oriental sore is present in various localities of Palestine.
2. There are locally acquired cases in various parts of Palestine.
3. The clinical history of the cases noted and the distribution of the cases and the lesions can be explained on the hypothesis that sandflies carry the disease.



## EXPLANATION OF PLATE XXX

- Fig. 1. *Leishmania ulcerosa*. From Jerusalem.
- Fig. 2. *Leishmania verrucosa gigantea*. From Baghdad.  
Size of lesion  $13\frac{1}{2}$  cm. Ulcerated area  $8\frac{1}{2}$  cm.



FIG. 2



FIG. 1







## EXPLANATION OF PLATE XXXI

Fig. 1. *Leishmania ulcerosa*. From Beth-Djalla (Palestine).

Fig. 2. *Leishmania nodosa*, From Beth-Djalla (Palestine).



FIG. 2



FIG. 1





## EXPLANATION OF PLATE XXXII

*Leishmania ulcerosa* on the trunk. From Persia (or Baghdad).





# A NOTE ON THE HISTOPATHOLOGY OF A CASE OF EXPERIMENTAL CUTANEOUS LEISHMANIASIS

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## PLATE XXXIII

The pathology of oriental sore has been frequently described, and in general the lesion has been considered to consist essentially of an infiltration in the corium of endothelial cells, plasma cells and lymphocytes. Changes in the epidermis which are entirely secondary to the infiltration of the corium have also been described. The histopathology of the following case of experimental Leishmaniasis is of interest for the following reasons :

1. The lesion occurred in the subcutaneous tissue.
2. Histologically the lesion was practically indistinguishable from tuberculosis.

### *History of the case.*

Male *aet.* 27, inoculated 9.9.25 on two points on the left forearm with *Herpetomonas* from a naturally infected sandfly, *Phlebotomus papatasi*. The sandfly was caught in Jericho, 8.9.25, and dissected 9.9.25, in Jerusalem, the inoculation being performed immediately after dissection.

In January, 1926, the patient noted a hard nodule under the site of one of the inoculated points. The nodule was subcutaneous, not attached to the skin or deep tissue, and grew rapidly in size, being, on 14.4.26, 9 mm. in length by 6 mm. in breadth. Puncture, on 14.4.26, of the nodule through the skin revealed numerous Leishman-Donovan bodies. Cultures of *L. tropica* were obtained from the lesion on the following modification of Noguchi's *Leptospira*



medium, Agar 1 part, Locke's solution made up with 0.2 per cent. glucose 8 parts, and fresh rabbit serum 1 part. The nodule was frequently punctured between 14.4.26 and 18.7.26, and it was found that the numbers of the parasites decreased; and smears made on 18.7.26 revealed no parasites, but cultures made on 18.7.26 and 19.7.26 were positive. By the middle of June, 1926, the nodule became attached to the skin, this attachment being due to trauma caused by frequent examinations, as will be shown later. It is interesting to note that although the skin was frequently punctured and parasites entered the puncture wound, no cutaneous lesion developed.

On 2.8.26 an incision through the skin above the nodule was made, one-half of the nodule together with the overlying skin was removed for histology and the other half was left *in situ*. The nodule was found to be 4.5 mm. in cross section.

Sections showed that the distance between the outer surface of the epidermis and the nearest point on the nodule was 2.5 mm., i.e., well out of reach of the proboscis of *Phlebotomus papatasi* which penetrates only about  $300\mu$ , i.e., about three-quarters of the length of the piercing parts are introduced into the skin. This explains why feeding experiments with *P. papatasi*, performed at a time when parasites were very numerous, all gave negative results.

The lesion itself was not encapsuled and consisted of a conglomeration of typical tubercles separated from each other by strands of connective tissue which were themselves infiltrated with lymphocytes, plasma cells, and endothelial cells. The tubercles were of varying stages of development, some recently formed, others showing advanced fibrosis and several caseation (Pl. XXXIII, fig. 1). The oldest lesions, i.e., those showing caseation, were not in the centre of the nodule, but were placed infra-laterally about 4.5 mm. below the surface of the epidermis. Since in performing the experiment the skin was scarified and the *Herpetomonas* were inoculated into the scarified points, the flagellates could have reached 4.5 mm. below the surface of the epidermis either by penetrating through the tissues or by being carried by the blood stream and settling in the endothelial cells of a neighbouring capillary—probably the latter, for *Herpetomonas* shows no capacity for active penetration. It appears that the

pathological process in all cases of cutaneous Leishmaniasis commences in the endothelial cells of a capillary, for sections show that infiltration of endothelial cells are usually round a capillary and, further, parasites are found in endothelial cells lining capillaries. In the present case a focus consisting of endothelial cells and plasma cells, the former being stuffed with parasites, was found near the centre of the lesion (Pl. XXXIII, fig. 4). In other parts the parasites were either absent or too few to be diagnosed histologically with certainty.

The epidermis was normal and the corium showed widely-scattered areas of infiltration consisting mainly of fibro-blasts with some small round cells and plasma cells. These infiltrated areas were not continuous with the main lesion from which they differed histologically in the absence of endothelial cells and giant cells which were such a marked feature of the main lesion ; they could, therefore, only have been caused by the trauma incidental to the frequent examinations.

## EXPLANATION OF PLATE XXXIII

Fig. 1. Section through lesion.  $\times 16$ .

(a) Scattered areas of infiltration in corium.

(b) Tubercles.

(c) Large caseating tubercle.

Microphotograph in two parts.

Fig. 2. Portion of a tubercle showing giant cells.  $\times 370$ .

Fig. 3. Portion of a tubercle showing giant cells.  $\times 85$ .

Fig. 4. Endothelial cell in centre of lesion containing numerous Leishman-Donovan bodies.  $\times 750$ .



FIG. 1



FIG. 2



FIG. 3



FIG. 4



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